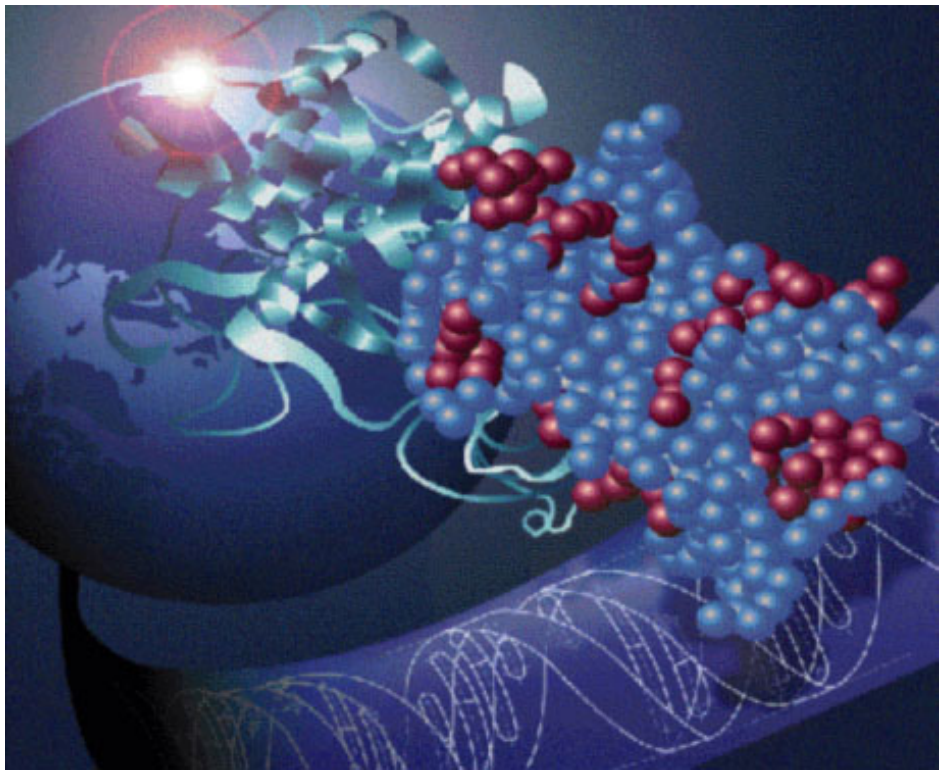


PROCISE®, PROCISE® cLC, and PROCISE® C Protein Sequencing Systems

User Guide

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

Part Number 4340645 Rev. A
02/2003

Contents

Preface

Safety and EMC Compliance Information

Chapter 1 Introduction

Chapter 2 Preparing the System

Chapter 3 Preparing and Running Samples

Chapter 4 Optimizing Sequencer Processes

Chapter 7 Maintenance

Appendix A Troubleshooting Guide

Appendix B Chemistry Overview

Appendix C Valves, Functions, Cycles, and Methods

Appendix D Warranty and Service Information

Index

Preface

How to Use This Guide

Purpose of This Guide

The Applied Biosystems *PROCISE®, PROCISE® cLC and PROCISE® C Protein Sequencing Systems User Guide* provides information on how to set up, prepare samples, run, and maintain the *PROCISE®* Protein Sequencing Systems.

Audience

This guide is intended for novice and experienced protein sequencer users. This manual is written for technicians, scientists, and researchers who wish to sequence polypeptide chains using the PROCISE and PROCISE cLC N-Terminal Sequencing System, and PROCISE® C-Terminal Sequencing System. It is designed to be a resource for everyone using the instrument, from beginning to experienced users.

Assumptions

This manual assumes that you are familiar with the following:

- Basic Microsoft® Windows® 2000 operations such as using the mouse, choosing commands, working with windows, and using the Windows 2000 computer hierarchical file system
- A general understanding of hard drives and data files
- Understanding of protein sample isolation and preparation
- Familiarity with Applied Biosystems N- or C-terminal sequencing chemistry

Conventions Used in This Guide

This guide uses the following conventions to make text easier to understand. For example:

- **Bold** indicates user action. For example:

  Type **0** and press **Enter** for the remaining fields.
- *Italic* text denotes new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

  Right-click the sample row, then select **View Filter > View All Runs**.

Computer Vocabulary and Operations

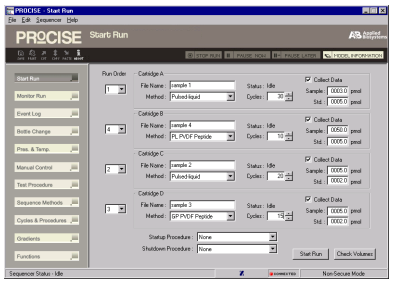
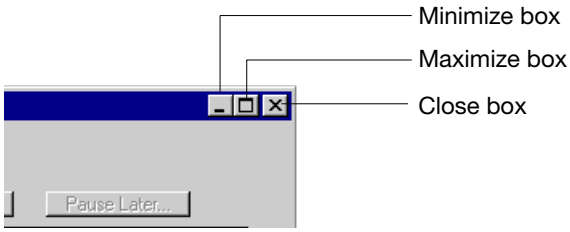
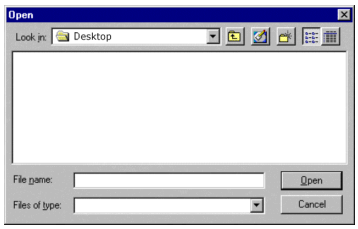
You should be familiar with the following basic computer vocabulary and operations:

Vocabulary and Operations	Description
Using the mouse	Clicking and double-clicking, selecting, and dragging.
Choosing commands	Using pull-down and pop-up menus, dialog boxes, radio buttons, and checkboxes.
Working with windows	Opening and closing, resizing and repositioning, scrolling, and understanding the active window.
Using the Microsoft Windows hierarchical file system	Finding files and creating folders.

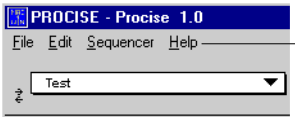
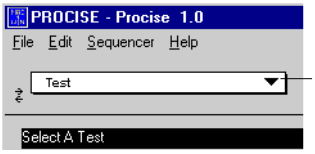
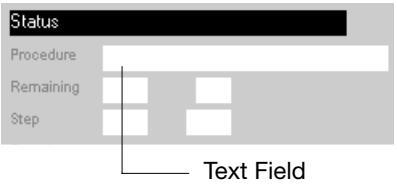
Windows 2000 Terms Used in This Manual

The following terms are used frequently in this manual to describe how to use *PROCISE* software.

Terms Used in This Manual:

Term	Description
Windows 	Display information and allow you to edit or enter additional information. If many windows are open, click one to make it active. When a window is active, you can click the top border, hold down the mouse button, and drag the window to another location on the screen. When you are finished working with a window, click the Close box to remove the window from the screen, or click another window. 
Dialog boxes 	Appear when you must make a decision or enter information. All other actions on the monitor screen are suspended until you close the dialog box by clicking a button, such as **Cancel**, **OK**, **Open**, or **Done**.

Terms Used in This Manual: *(continued)*

Term	Description
Menus 	Provide access to various functions you can perform with the software. A heavy arrow (▶) after a menu item indicates that a submenu appears when you click that choice and hold down the mouse button.
Pop-up menus 	Display a heavy arrow (▼) and are found in dialog boxes or windows. When you click a pop-up menu and hold down the mouse button, a submenu appears. These menus allow you to choose dialog box entries from item lists.
Text fields 	Rectangular areas in which you can enter information. Click in an entry field to display a cursor, and use the keyboard to enter the information.
Buttons 	Rectangles with rounded corners that allow you to accept or cancel the contents of a dialog box or perform functions (such as printing) within the dialog box. A button with a heavy outline is the default button that applies if you press the **return** key.
Radio buttons 	Small circles that appear in front of choices. A black dot appears in the center of the circle to indicate that the associated option is selected.
Checkboxes 	Boxes that you click to select certain options in a dialog box. Depending on the version of your operating system, an **X** or √ appears in a checkbox to indicate that the associated option is selected.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well microtiter plate.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page iv-xv.

How to Obtain More Information

Related Documentation

The following related documents are shipped with the system:

- *PROCISE®, PROCISE® cLC and PROCISE® C Protein Sequencing Systems User Guide User Guide* – Describes how to set up, prepare samples, run and maintain the instrument.
- *PROCISE and PROCISE cLC Protein Sequencer Site Preparation and Safety Guide* (P/N 4314377) or *PROCISE C Protein Sequencer Site Preparation and Safety Guide* (P/N 431660) – Describes the site, electrical, and laboratory requirements of the instrument system so that you can prepare the site before installation.
- PROCISE ***Online Help*** **–** Describes the PROCISE Software PC Version 2.0 and provides procedures for common tasks. Help is available from the Help menu or by pressing F1.

Some supportive information to your system are the following references:

Title	P/N
140C Microgradient Delivery System User's Manual	903078
140D Microgradient Delivery System User's Manual	903586
Your Windows 2000 Workstation Operating System Online Help	—

Note: For additional documentation, see "How to Obtain Services and Support" on page xiii.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Services and Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Services and Support**.

At the Services and Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Services and Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Safety and EMC Compliance Information

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action, as described below:

IMPORTANT! Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Indicates a potentially hazardous situation that, if not avoided, can result in minor or moderate injury. It can also alert against unsafe practices, damage to an instrument, or loss of data.

Indicates a potentially hazardous situation that, if not avoided, can result in serious injury or death.

Indicates an imminently hazardous situation that, if not avoided, will result in serious injury or death. This signal word is to be limited to the most extreme situations.

Examples

Examples of the safety alert words appear below:

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well microtiter plate.

Do not touch the lamp. This can damage the lamp.

CHEMICAL HAZARD. Formamide is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth-defect hazard. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on Instruments

Electrical Symbols

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see “Safety Labels on Instruments” on page iv-xvii). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this document.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Safety Labels on Instruments

The following Caution, Warning, and Danger statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

English	Francais
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangeureux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	**ATTENTION** Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
DANGER Laser radiation present when open and interlock defeated. Avoid direct exposure to laser beam.	**DANGER** Rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de sécurité. Eviter toute exposition directe avec le faisceau.
DANGER Laser radiation when open. Avoid direct exposure to laser beam.	**DANGER** Rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.

General Instrument Safety

WARNING **PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and Lifting the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and Lifting Stand-Alone Computers and Monitors

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the device is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before actually lifting and carrying.
- Instead of lifting the computer or the monitor from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs).

Chemical Safety

Chemical Hazard Warnings

WARNING CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. See "About MSDSs" on page iv-xix.
- Minimize contact with chemicals. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing. For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, a fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the cleanup procedures recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

CHEMICAL WASTE HAZARD. Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the MSDSs for the chemicals in a waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers

  A primary waste container holds the immediate waste. A secondary container contains spills or leaks that may occur from the primary container. Both must be compatible with the waste material and meet national, state/provincial, and local requirements for container storage.
- Minimize contact with and inhalation of chemical waste. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing.
- Handle chemical wastes in a fume hood.
- After you empty a chemical waste container, seal it with the cap provided.
- Dispose of the contents of a waste container in accordance with good laboratory practices and local, state/provincial, and/or national environmental and health regulations.

About Waste Profiles

A waste profile is provided with this instrument and is contained in the *PROCISE and PROCISE cLC Site Preparation and Safety Guide* and *PROCISE C Protein Sequencing System Site Preparation and Safety Guide.* Waste profiles list the percentage compositions of the reagents within the waste stream at installation and the waste stream during a typical user application. These profiles assist users in planning for instrument waste handling and disposal, which must be in accordance with local, state/provincial, or national regulations. Read the waste profiles and all applicable MSDSs before handling or disposing of waste.

IMPORTANT! Waste profiles are not a substitute for MSDS information.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

Note: Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical Safety

Shock Hazards

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

ELECTRICAL SHOCK HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the PROCISE, PROCISE cLC or PROCISE C protein sequencer, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power Supply

ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

ELECTRICAL HAZARD. A short circuit can result from working on an instrument when the power supply is operating. To avoid a short circuit, turn off the instrument before servicing the instrument.

Physical Hazard Safety

Ultraviolet Light Sources

WARNING **ULTRAVIOLET LIGHT HAZARD.** Exposure to ultraviolet radiation can cause blindness or permanent eye damage. To prevent eye injury, change the detector sensitivity from the ultraviolet to the visible range (520 nm) before beginning any detection or maintenance procedures. Always wear protective UV-absorbing glasses when looking into the detector. Turn off the lamp power before removing it from its fixture.

WARNING **ULTRAVIOLET LIGHT HAZARD.** Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing.

Compressed Gases

WARNING **PHYSICAL HAZARD**. **Nonflammable compressed gas** (argon)**.** Contents are under pressure. Receive proper training on the handling of compressed gases before use. Exposure to rapidly expanding gas may cause frostbite. High concentrations of vapors in the immediate area can displace oxygen and cause asphyxiation. Use only in areas with adequate ventilation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **EXPLOSION HAZARD.** Pressurized gas cylinders are potentially explosive and can cause severe injury if not handled properly. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

Solvents and Pressurized Fluids

WARNING **PHYSICAL INJURY HAZARD.** Always wear eye protection when working with solvents or any pressurized fluids.

WARNING **PHYSICAL INJURY HAZARD.** To avoid hazards associated with high-pressure fluids in polymeric tubing:

- Be aware that PEEK™ tubing is a polymeric material. Use caution when working with any polymer tubing that is under pressure.
- Always wear eye protection when in proximity to pressurized polymer tubing.
- Extinguish all nearby flames if you use flammable solvents.
- Do not use PEEK tubing that has been severely stressed or kinked.
- Do not use PEEK tubing with tetrahydrofuran or concentrated nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause PEEK tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing. Electrical sparks may result.

Biological Hazard Safety

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4, http://bmbl.od.nih.gov)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR §1910.1030, http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).

Additional information about biohazard guidelines is available at:
http://www.cdc.gov

Computer Workstation Safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

- Use equipment that comfortably supports the user in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian Safety Standards
- Canadian EMC Standards
- European Safety and EMC Standards
- Australian EMC Standards

U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 3101-1, “Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements.”

This instrument has been tested to and complies with standard CSA 1010.1, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements.”

Canadian EMC Standards

This instrument has been tested to and complies with standard ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.

European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1, “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements” and EN 61010-2-010, “Particular Requirements for Laboratory Equipment for the Heating of Materials.”

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), “Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements.”

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, “Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment.”

Introduction 1

This chapter describes the contents of this manual, and provides a general system description of the PROCISE® Protein Sequencing Systems, reagent lists, and user management features.

The following topics are contained in this chapter:

About This Manual

Manual Organization

This user's manual provides detailed instructions on the use and maintenance of the PROCISE Protein Sequencing Systems. The manual is organized into the following chapters and appendixes:

Chapter/Appendix	Contents
Chapter 1 Introduction	Contains a brief system description, reagent and solvent listings and information on user management features.
Chapter 2 Preparing the System	Describes how to prepare the PROCISE Protein Sequencing Systems with reagents, HPLC pump, and the UV detector for a run.
Chapter 3 Preparing and Running Samples	Describes how to prepare the PROCISE Protein Sequencing Systems samples, perform tests, and start a run.
Chapter 4 Optimizing Sequencer Processes	Describes how to optimize sensor functions, the injector percentage, flask dry times, protein sequencer chemistry, and PTH-amino acid separation to provide consistent chromatography.
Chapter 5 Troubleshooting Guide	Provides instructions for troubleshooting problems you may encounter while using the PROCISE Protein Sequencing Systems.
Chapter 6 Custom Functions, Cycles, Methods and Gradients	Provides instructions on creating custom functions, cycles, methods, and gradient programs to improve sample sequencing results.
Chapter 7 Tests and Procedures	Includes instructions for running the tests and procedures included with this system.
Chapter 8 Maintenance	Contains recommendations and instructions for the routine maintenance, repair, and replacement of system components.
Appendix A Chemistry Overview	Describes both the N-terminal and C-terminal sequencing chemistry.
Appendix B Valves, Functions, Cycles, and Methods	Describes the types of valves, and the purpose of functions, cycles, and sequencing methods used by the PROCISE Protein Sequencing System.
Appendix C	Contains the warranty statement.
User Bulletins	Serves as a placeholder for user bulletins that may be issued by Applied Biosystems for these systems.

System Description

System Overview

The PROCISE Protein Sequencing Systems:

- Sequentially cleave amino acids from the N-terminus of a protein or peptide (The PROCISE C sequencer cleaves amino acids from the C-terminus of a protein or peptide)
- Separate and identify the cleaved amino acids
- Analyze the data

Cleavage and separation of the amino acids occur during what is commonly referred to as a sequencing run. The following section includes a brief description of:

- What occurs during a sequencing run
- The main system components:
  - PROCISE Protein Sequencer
  - ABI 140C or 140D Microgradient Delivery System (the pump)
  - UV/VIS Detector (the detector)
  - A Windows® 2000–based computer

The system and the protein sequencer are sometimes referred to as the 49X where X represents the number of reaction cartridges on the protein sequencer.

The protein sequencer will have one (model 491), two (model 492) or four (model 494) reaction cartridges.

The Sequencing Run

To execute a sequencing run, your sample is first applied to a solid support, such as a PVDF membrane or a glass-fiber disk. The sample on the support is then placed inside one of the reaction cartridges on the protein sequencer. During a run, a sequential degradation is carried out inside the reaction cartridge. In each degradation cycle, the N- (or C-) terminal amino acid is derivatized and cleaved from the protein or peptide.

The derivatized amino acid is then transferred from the reaction cartridge to the flask on the protein sequencer. In the PROCISE or PROCISE cLC sequencer, the ATZ-amino acid is converted to a more stable phenylthiohydantoin-amino acid (PTH-AA). The PTH-AA is then transferred from the flask to the injection valve for subsequent injection, separation and quantitation on the chromatographic system. In the PROCISE C sequencer the alkylated thanhydantoin amino acid (ATH-AA) is dried in the flask, then transferred from the flask to the injection valve for subsequent injection, separation, and quantitation on the chromatographic system.

The PROCISE Protein Sequencer

The protein sequencer controls precise delivery of up to twelve different solvents and reagents. Solvents and reagents are transferred to and from the reaction cartridge, the flask, and the sample injection loop by a microprocessor-controlled, electromechanical, pressure-driven chemical delivery system.

Chromatographic Components

The chromatographic components of this system used to detect the derivatized amino acids (PTH-AAs of ATH-AAs) are:

- The ABI 140C or 140D pump—a dual-syringe, programmable liquid chromatography system
- The UV/VIS detector—a low-noise, high-sensitivity, variable wavelength UV/VIS detector
- A reversed-phase analytical column in a temperature-controlled heating block that separates the derivatized amino acids

Because the different amino acid derivatives have unique relative affinities for the column, each amino acid derivative exits the column at different times.

The Computer

The computer controls and monitors the PROCISE cLC Protein Sequencing System. The computer is equipped with two types of software:

- PROCISE control software
- SequencePro™ Data Analysis Application software

Control Software

The PROCISE control software controls and coordinates the operation of all the instruments in the system. The software also constantly monitors each sequencing run and overall system operation.

Standard automated functions, cycles, sequencing methods and gradient programs are included in this software. You can select various combinations of cycles, methods and gradients for sequencing runs.

In addition, you can create custom functions, cycles, methods and gradients as described in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients.”

SequencePro Software

The SequencePro software analyzes and reports protein and peptide sequence data.

The output from the UV/VIS detector is collected by the PROCISE control software. A 24-bit analog-to-digital (A/D) converter is located inside the protein sequencer. The A/D converter converts the analog signal to a digital signal and transmits the digital signal to the SequencePro software.

Refer to the SequencePro software user’s manual for more information on this product.

Basic System Connections

Instrument Connections

During installation, all the physical connections between the instruments in this system are made by your Applied Biosystems Service Representative.

Note: If the system is moved, or is shut down for an extended period of time, review this section to ensure that all connections are properly made before restarting the system.

Electrical Connections

Four power connections are required for the PROCISE Protein Sequencing System:

- The PROCISE Protein Sequencing System has an automatic line-switching power supply, and will operate between 90 and 264 VAC at a frequency of 50 or 60 Hz.
- The computer is equipped with an automatic switching power supply, and will operate between 90 and 264 VAC at a frequency of 50 or 60 Hz.
- The ABI 140C or 140D (the pump)
- The UV detector

The ABI 140C or 140D pump and the UV Detector are shipped from Applied Biosystems with the voltage set for 120 VAC. However, the system is shipped with a universal voltage kit that contains the fuses and power cords necessary to reconfigure these instruments for most other voltage requirements (100, 120, 220, or 240 VAC).

Note: We recommend a dedicated electrical line with a circuit breaker for this system. The outlet must be located within 2.5 m (8 ft) of the system.

Additional connections may be needed for optional modules, such as a chart recorder. Refer to the *PROCISE and PROCISE cLC Site Prep and Safety Guide* (P/N 4314377) for more information.

Communication Connections

To connect the system connections:

1. Connect the protein sequencer to the serial port on the computer.
2. Connect the printer to the printer port on the computer.
3. Connect the HPLC pump to the pump port on the sequencer.
4. Connect the signal output from the UV/VIS detector to the detector input port on the sequencer.
5. (Optional) To use a chart recorder:

If...	Then...
you use a chart recorder with an external paper feed control	1. Connect the respective pins to the two terminals marked Event 1 on the rear connection strip of the pump. 2. Set the chart recorder to auto-paper feed with a chart speed of 5 mm/min.

For an illustration of the instrument connections in this system, see Figure 1-1 on page 1-6.

Instrument Connections

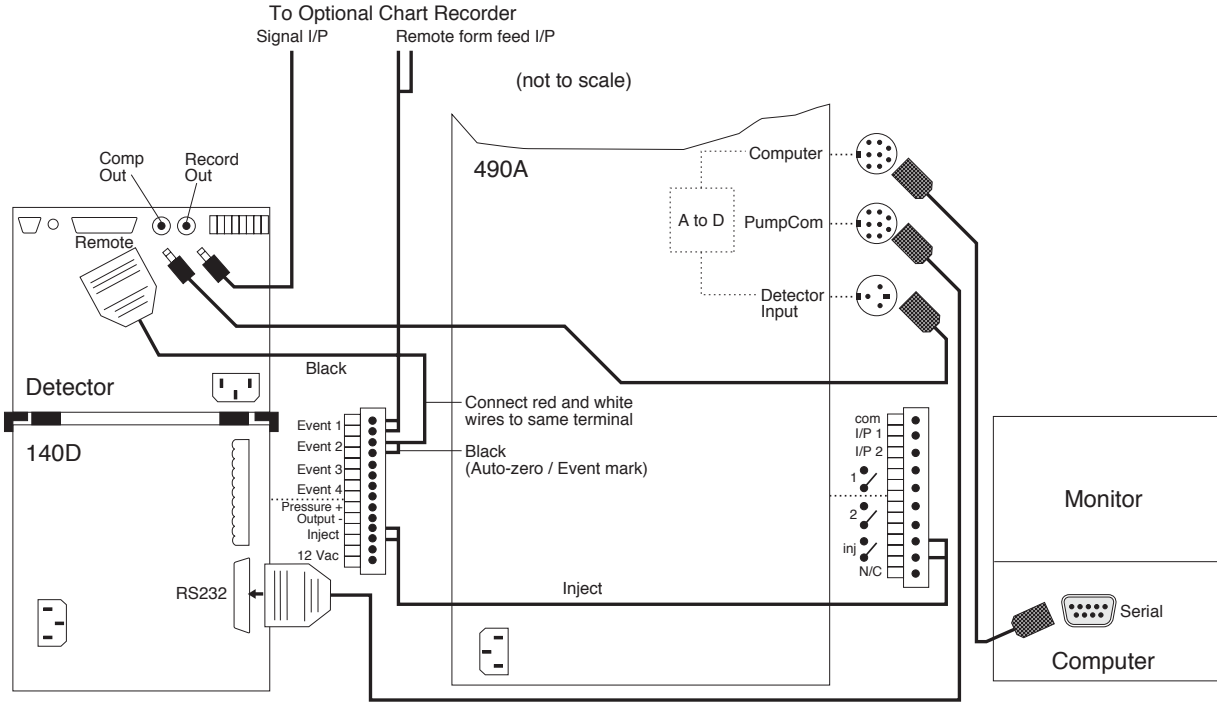


Figure 1-1 Instrument connections

Argon Supply Connections

Note: If the input pressure drops below 60 psi during sequencing, the system will pause.

Requirements	Specifications
Minimum of 1 Argon cylinder	• Size 1A • Pre-purified; 99.998% purity or greater
Regulator	• One for each cylinder • Swagelok-type end fittings on exit side for connection to 1/4-in. (6.355 mm) o.d. tubing. • Set between 65 and 75 psi (448 and 517 kPa)
CGA 580 cylinder-adaptor	One for each cylinder

WARNING **EXPLOSION HAZARD.** Ensure that the pressurized gas cylinder is safely attached to the wall or cylinder truck by means of approved brackets or clamps. Failure to do so could cause the cylinder to fall over and explode, which could cause physical hazard. Always turn off, cap, and secure any cylinder that is not in use. Keep cylinders away from electrical circuits and excessive heat.

System Plumbing Diagram

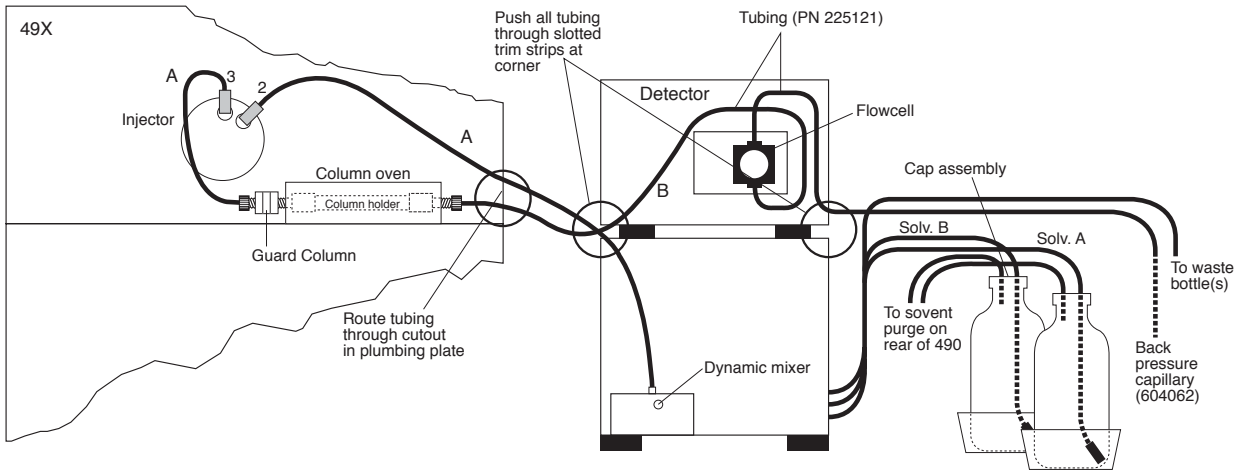


Figure 1-2 PROCISE cLC plumbing diagram

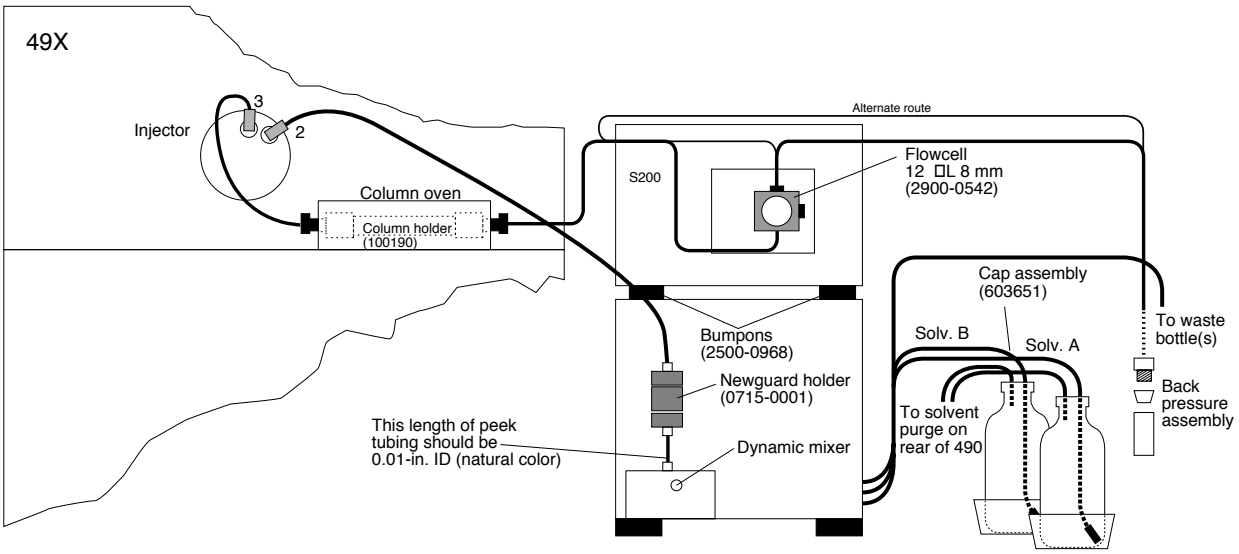


Figure 1-3 PROCISE and PROCISE C plumbing diagram

Reagents and Solvents Used on the PROCISE System

Table of Chemical Storage Conditions

All reagents and solvents supplied by Applied Biosystems are highly purified and sequencer-tested to ensure optimal performance. Table 1-1 contains a list of reagents and solvents used with the standard sequencer cycles.

Table 1-1 Chemicals used on the PROCISE protein sequencer

Bottle Position	Chemical Contents	Part Number	Storage Conditions
R1	R1, 5% phenylisothiocyanate (PITC) in heptane	400208	–20 °C [a]
R2	R2C, N-methylpiperidine/butanol/isopropanol/water	4310689	4 °C [a]
R3	R3, Trifluoroacetic acid (TFA), neat	400003	RT [b]
R4	R4A, 25% TFA in water, with 0.01% dithiothreitol (DTT)	400028	4 °C [a]
R5	Acetonitrile/N-Acetylcysteine	4340966	RT [b]
S1	S1, n-heptane (not used in standard cycles)	400079	RT [b]
S2	S2B, ethyl acetate	400854	RT [b]
S3	S3, n-butyl chloride	400008	RT [b]
S4	S4B, 20% acetonitrile in water	400314	RT [b]
X1	Not used in standard cycles	–	–
X2	Not used in standard cycles	–	–
X3	Not used in standard cycles	–	–
—	PTH Amino Acid Standard Solution	4340968	RT [b]
—	Biobrene Plus	400385	4 °C [a]
—	ß-lactoglobulin	400979	4 °C [a]

a. Allow these items to reach room temperature before opening. When these bottles are opened while still cold, water can condense inside. Check bottle caps for tightness after placing these bottles at either 4 °C (2–8 °C) or –20 °C (–15 to –20 °C).
b. RT (Room Temperature) = 15–20 °C in a dark, dry place.

Supporting Chemicals

Supporting solvents and chemicals needed to run the system are listed in Table 1-2.

Table 1-2 Solvents and chemicals supporting the PROCISE Systems

Chemicals	Part Number	Storage Conditions
Solvent A3, 3.5% THF Tetrahydrofuran in Water, 1 L (PROCISE)	401464	RT
Solvent B2, 12% Isopropanol and Acetonitrile, 1 L (PROCISE)	401570	RT
Solvent B (not used),100% acetonitrile, 1 L	400313	RT
Acetone (HPLC grade)		RT
MeOH (Methanol)	400470	RT
Premix Buffer Concentrate	401446	4 °C

Reagents and Solvents Used on the PROCISE cLC System

Table of Chemical Storage Conditions

All reagents and solvents supplied by Applied Biosystems are highly purified and sequencer-tested to ensure optimal performance. A list of reagents and solvents used with the standard protein sequencer cycles is given in Table 1-3.

Table 1-3 Chemicals used on the PROCISE cLC Protein Sequencer

Bottle Position	Reagent/Solvent	Part Number	Storage Conditions
R1	5% phenylisothiocyanate (PITC) in n-heptane—40 mL	400208	–20 °C[a]
R2	R2C, N-methylpiperidine/butanol/isopropanol/water —40 mL	4310689	4 °C[a]
R3	(TFA), neat—40 mL	400003	RT[b]
R4	25% trifluoroacetic acid (TFA) in water with 0.01% dithiothreitol (DTT)—40 mL	400028	4 °C[b]
R5	Acetonitrile/N-Acetylcysteine	4340966	RT[b]
S1 & S3	S3, n-butyl chloride—2 bottles, 200 mL each	400008	RT[b]
S2	S2B, ethyl acetate—450 mL	400854	RT[b]
S4	S4C, 10% acetonitrile in water—200 mL	402051	RT[b]
X1	Not used in standard cycles	400470	–
X2	Not used in standard cycles	–	–
X3	Not used in standard cycles	–	–
—	PTH- Amino Acid Standard Solution	4340968	RT[b]
—	Beta-lactoglobulin Sequencing Standard	400979	4 °C[a]
—	BioBrene™ Plus	400385	4 °C[a]

a. Allow these chemicals to reach room temperature before opening. If these bottles are opened while still cold, water can condense inside. Check bottle caps for tightness after placing these bottles at either 4 °C (2 to 8 °C) or –20 °C (–15 to –20 °C).
b. RT (Temperature) = 15–20 °C in a dark, dry place.

Supporting Chemicals

Table 1-4 Supporting chemicals

Chemicals	Part Number	Storage Conditions
Solvent A3, 3.5% THF Tetrahydrofuran in Water, 450 mL	401887	RT
Solvent B2, 12% Isopropanol and Acetonitrile, 450 mL	401886	RT
Acetone (HPLC grade)	–	RT
MeOH (Methanol)	400470	RT
Premix Buffer Concentrate	401446	4 °C

Reagents and Solvents Used on the PROCISE C System

Sequencing Chemicals

Figure 1-4 illustrates the positions of the bottles on the protein sequencer.

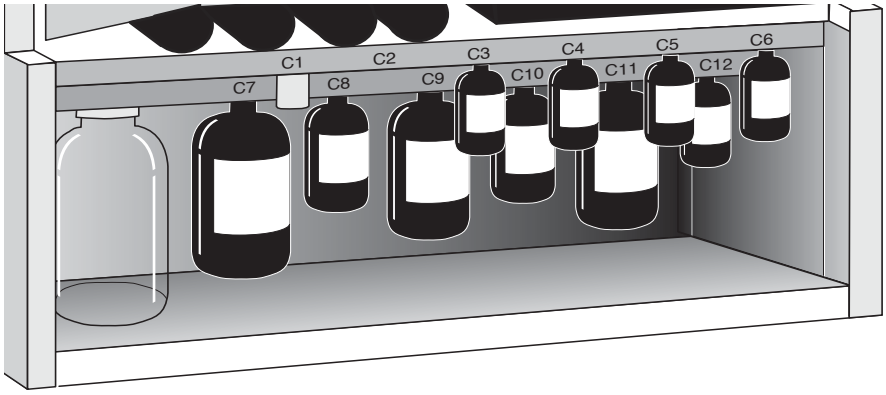


Figure 1-4 PROCISE C protein sequencer bottle configuration

All reagents and solvents supplied by Applied Biosystems are highly purified and tested to ensure optimal performance. Table 1-5 lists the reagents and solvents used for the standard sequencer cycles.

Table 1-5 PROCISE C Reagents, Solvents, and Standards

Bottle Position	Reagent/Solvent	Volume (mL)	Part Number	Storage Conditions
C1	BOC Methylnapthylthiohydantoin–Amino Acid Standard	—	403088	–20 °C[a]
C2	Empty	—	—	—
C3	N-methylimidazole/acetonitrile	40	402141	RT[b]
C4	Piperidine thiocyanate/acetonitrile	40	401700	RT[b]
C5	Acetonitrile	40	402161	RT[b]
C6	Acetic anhydride/lutidine/acetonitrile	40	402142	RT[b]
C7	Ethyl acetate	450	402160	RT[b]
C8	Bromomethylnapthalene/acetonitrile	100	401703	4 °C[a]
C9	20% Acetonitrile in water	200	402162	RT[b]
C10	Tetrabutyl ammonium thiocyanate/acetonitrile	100	401854	RT[b]

Table 1-5 PROCISE C Reagents, Solvents, and Standards *(continued)*

C11	Diisopropylethylamine/heptane	200	401702	RT[b]
C12	Trifluoroacetic acid	50	401701	RT[b]
N/A	Horse Apomyoglobin Standard	—	402231	–20 °C[a]

a. Allow the chemicals to reach room temperature before opening. If the bottles are opened while cold, water can condense inside them. Check the bottle caps for tightness after placing these bottles at either 4 °C (2 to 8 °C) or –20 °C (–15 to –25 °C).
b. RT (Room temperature) = 15 to 20 °C in a dark, dry place.

Accessing the PROCISE Control Software

PROCISE® PC 2.0 software can be installed in two different modes:

- Regulatory
- Non-regulatory

Regulatory Mode

The regulatory mode offers a set of user management features that allow an administrator to control access to the features of the PROCISE software. The administrator can:

- Create new users
- Create and edit user types
- Manage individual user access to specific screens or capabilities
- Deactivate users

When the software is installed in regulatory mode, users are required to log in to the software with an ID and password.The Event Log records the name of the user along with the time and date of the event. Creating or editing cycles, procedures, and methods requires verification (re-entry) of user ID and password in order for changes to be saved.

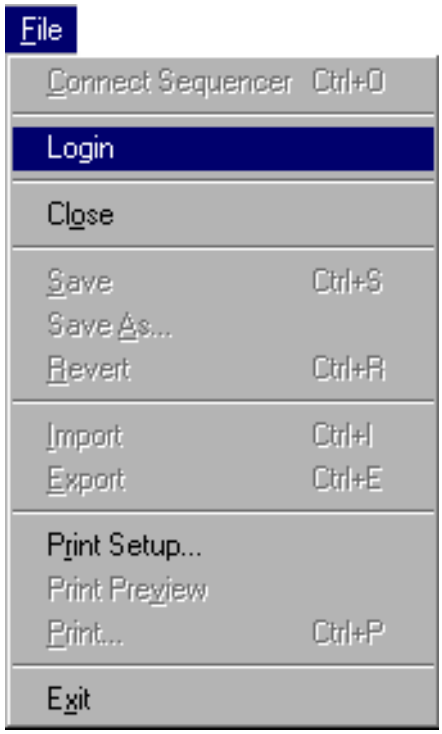
When you open the PROCISE software in the regulatory mode, you can view the Monitor Run and the Event Log screens without a user log in. Access to any other screens or any of the functionality of the PROCISE software requires the user to log in.

Regulatory Mode Login

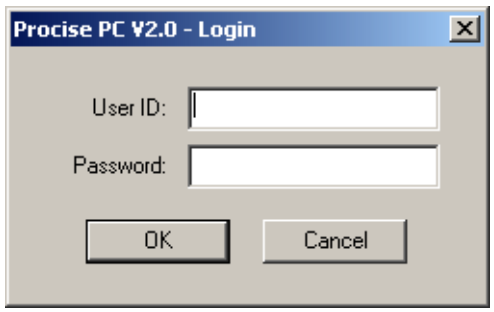
In the regulatory mode, all users must log in for access to PROCISE software functions.

To access the PROCISE software functions:

1. Select **File** >**Login**.



2. Enter the **User ID** and **Password**.



3. Press **OK**.
   All of the screens will now be accessible and the user's initial(s) and last name will appear in the lower right hand corner of the PROCISE screen.

While a user is logged in to the PROCISE software, the Event Log entries for the session will be identified with the user's full name, system time, and date stamp.

The initial user ID and password combination for all users is created by the PROCISE administrator. If a password is lost or forgotten, the administrator can create a new initial password for the user.

Note: After three failed attempts to log in to the software, the user ID/ password combination will be inactivated and the administrator will need to create a new initial password.

Logout

To minimize unauthorized use of the PROCISE software, you should logout after completing the necessary tasks.

To log out select **File > Logout**.

Immediately on logout, the PROCISE software returns to the Model Information screen. The Monitor Run and Event Log screens remain active so that the sequencer can be monitored. No user interaction with the sequencer is possible without a logon. The PROCISE software should be left in this mode when no user interaction is required.

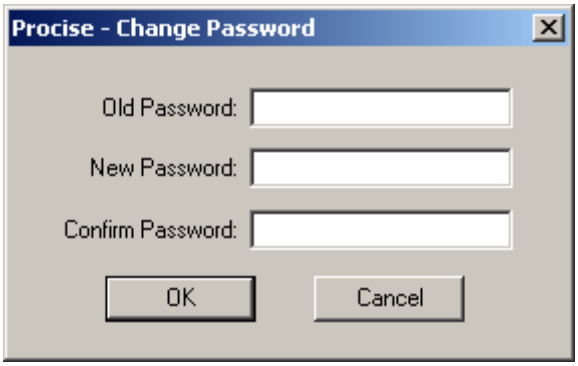
Note: Closing the PROCISE software while the sequencer is running causes the sequencer to pause. Chromatographic data is downloaded from the sequencer to the computer as it is collected. The sequencer memory only has capacity for 2 to 3 chromatograms. To avoid losing data, the sequencer pauses when the on-board memory is full.

User Changes Password

The first time you log in to the PROCISE software, you must create a new password. This password should be known only to you; do not share with the administrator or any other user.

To create a new password:

1. Log in to the PROCISE software to access the Change Password dialog box.



2. In the Change Password pop up menu enter the:
   a. Administrator-created initial (Old) password.
   b. New password twice, once in the New Password field and once in the Confirm Password field.
3. Click **OK** to exit the Change Password menu.

About Passwords

- Passwords must be a minimum of 6 and maximum of 15 alphanumeric characters.
- PROCISE passwords expire in nine months (or less if a shorter expiration is set by the PROCISE administrator). The PROCISE software begins prompting the user to create a new password two weeks prior to expiration of the current password.
- If the password expires before a new password is created, the PROCISE administrator will need to create a new initial password for the user.

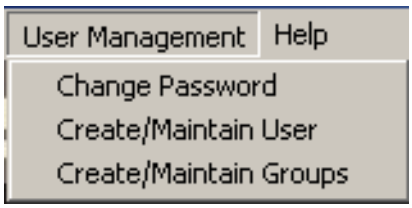
To access to the change password menu after log in, select **User Management > Change Password**. For users without administrative rights, the other user management selections will be unavailable.

Timeout

All users have a timeout setting. This feature automatically logs the user out after a specified period of inactivity. The length of the timeout interval is set by the PROCISE administrator. If the user is away from the system for longer than the timeout interval, the software logs the user out and the user will need to log on again to resume the session. Any pending actions not completed by a save, save as, delete or enter command before the timeout interval is exceeded will not be stored and will need to be repeated after log in.

Accessing User Management

The PROCISE administrator has access to the user management feature of the PROCISE software. The administrator can create new users, set password expiration and screen timeout durations, create and edit user types, manage individual user access to specific screens or capabilities as necessary and deactivate users.

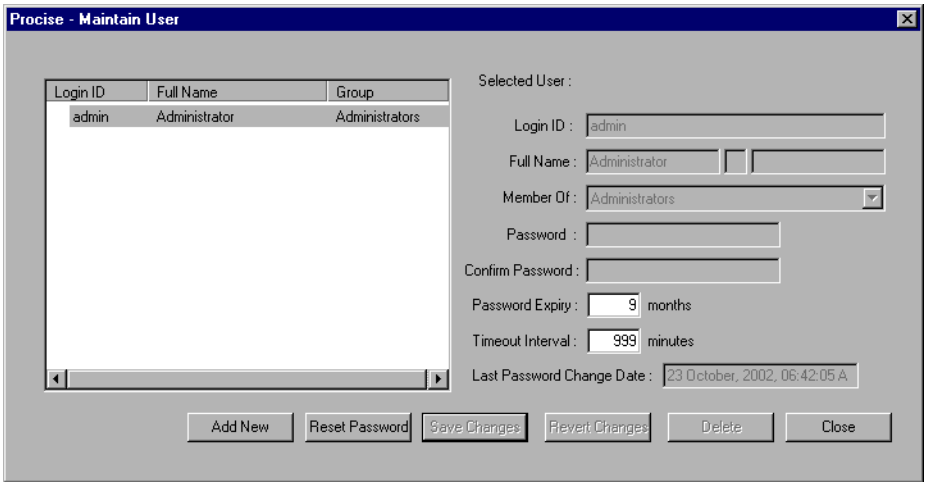


Create/Maintain User

The Create/ Maintain User window allows the administrator to manage user access to the PROCISE software.

To manage and add user access:

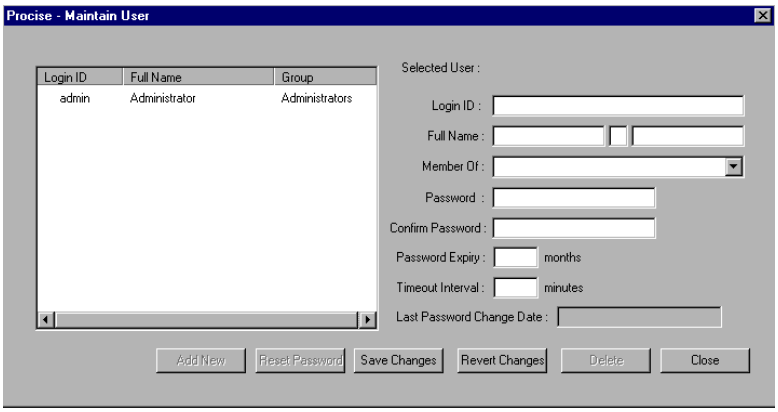
1. Select **User Management > Create/Maintain User**.



All fields will be grayed out except the Password Expiry and the Timeout Interval field. The Timeout Interval is the amount of idle time allowed before the software will log out a user. The default values are 9 months for password expiration and 999 minutes for the timeout interval. Custom default values can be set at this point or these values can be set individually as each user is set up.

The range for password expiration is one to nine months. The range for the timeout interval is 1 to 999 minutes.

2. Click **Add New** to activate all the entry fields.



3. Enter a Login ID for the new user. The Login ID can be any combination of 3 to 15 alphanumeric characters.

   Note: The full legal name of the user associated with the Login ID must be entered. The user's full name will be recorded with all actions reported to the Event Log during the session.

Default Groups and Access Rights

Assign the user to a group according to the access rights to be assigned to the user. Details of the access rights assigned to each group can be viewed in the Create/Maintain Groups screen. The default groups are:

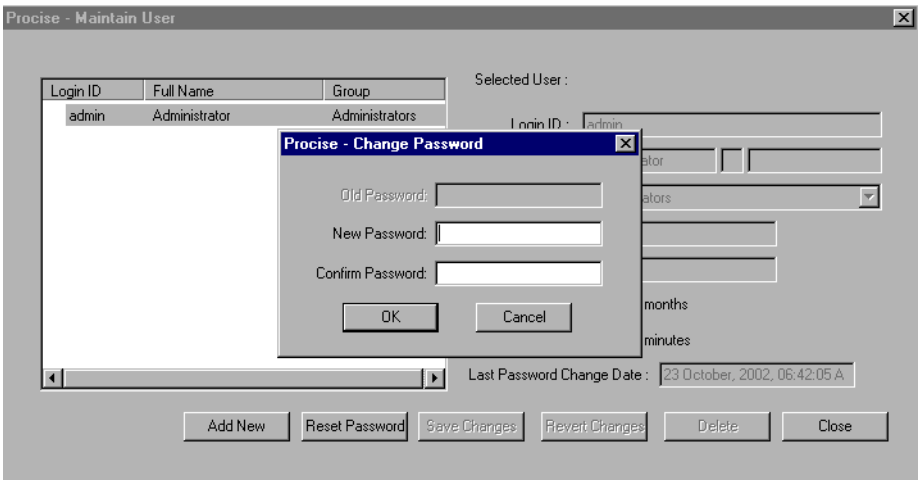
- Administrators - Access to all features and capabilities of the PROCISE software including full User Management.
- Power Users - Access to all features and capabilities of the PROCISE software except User Management.
- Editors - Same access as Power User except can not delete user functions, gradients, cycles, procedures and methods.
- Operators - Access to functions necessary to operate the PROCISE sequencer. Can not edit or delete user functions, gradients, cycles, procedures and methods.
- Viewers - Access to screens for viewing only. Can not operate the PROCISE sequencer.

To assign a user to a group:

1. Set and confirm an initial password for the user. Passwords must be a minimum of 6 and maximum of 15 alphanumeric characters.
2. Set password expiration and timeout interval if values other than the default values are required.
3. Click **Save Changes** to store the new user.
   Click **Revert Changes** to clear all entries.

To change the password for a user:

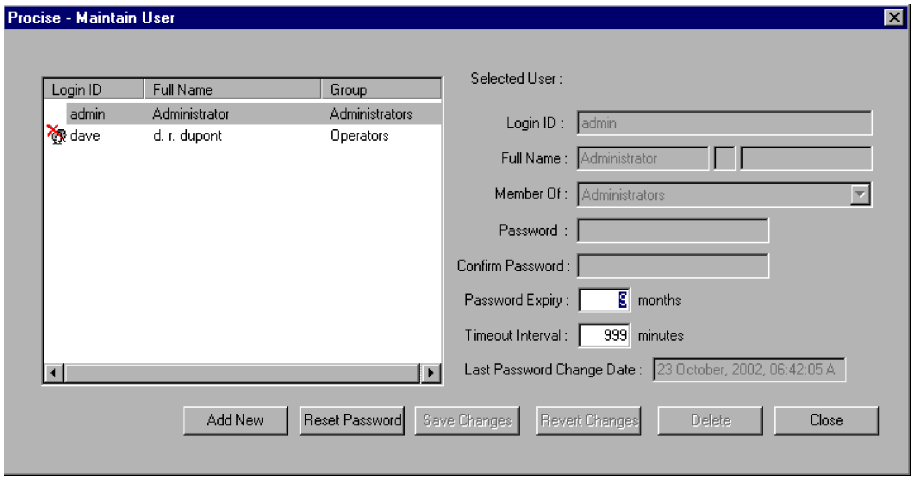
1. Select the user from the list at the left of the screen.
2. Click **Reset Password.**



3. Enter and confirm the new initial password.
   Passwords must be a minimum of 6 and maximum of 15 alphanumeric characters.

To inactivate a user:

1. Select the user from the list at the left of the screen.



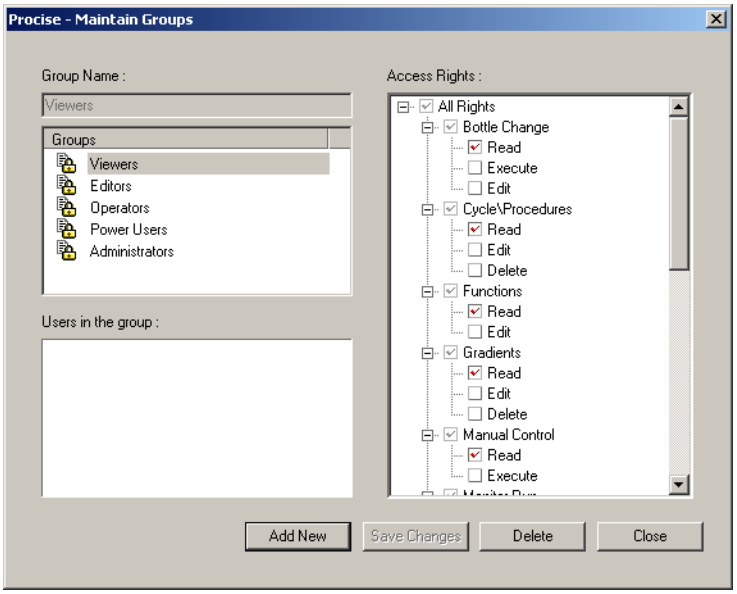
2. Click **Delete** and a dialog box will appear requiring confirmation.
3. Select **Yes** and the user will be marked as inactivated in the list but not removed from the list. The default administrator can not be inactivated.

Create/ Maintain Groups

The Maintain Groups window allows the administrator to manage group access to the PROCISE software.

To view group access rights and group members:

1. Select **User Management > Create/Maintain Groups**.
2. Highlight the group from the list at the upper left of the screen.
   The access rights for that group will be displayed in the scroll box on the right of the screen and the members of the group will be displayed on the lower left.



To add a new group:

1. Click **Add New**.
2. Enter a name for the new group.
3. Use the check boxes in the scroll box on the right of the screen to select the privileges to be given to the new group.
4. Provide access privileges. Click:
   - **Next** to a screen name to give full access to that screen.
   - On the specific function to provide limited access.
5. Click **Save Changes** when completed.

To delete a group:

1. Highlight the group from the list at the left of the screen.
2. Click **Delete** and a pop up requiring confirmation appears.
3. Select **Yes** to remove the group from the list.

   Note: Default groups can not be deleted.

Preparing the System 2

This chapter describes the process of preparing the PROCISE® Protein Sequencer Systems and the HPLC system for a run.

The following topics are contained in this chapter:

Preparing the PROCISE and PROCISE cLC System for a Run

Guidelines for Preparing Standards and Solvents

We strongly recommend you follow the guidelines listed below when preparing standards and other solutions for use on this system.

- Dedicate a low-traffic area in your lab for all solution preparation to make it easier to keep the preparation area and samples clean.
- Clean all work surfaces with undiluted methanol before preparing samples.
- Rinse pipette tips and all other glass receptacles with deionized water or solvent before use.
- Rinse all forceps and other tools and dry all tools before use.
- Always wear non-powdered gloves to prevent contamination of standards and other solutions.

Setting Protein Sequencer Pressures

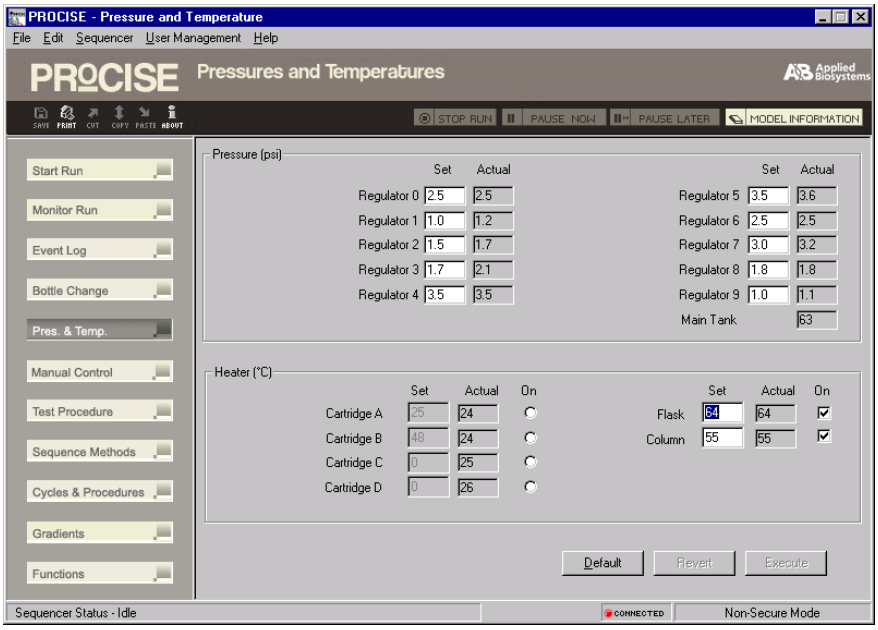
Follow these guidelines for setting pressures:

- If the protein sequencer loses pressure, or if the pressures and temperatures have been modified via functions such as the automatic leak test, click **Default** to restore the default settings.
- Pressures and temperatures for the protein sequencer are set and adjusted from the Pressures & Temperatures dialog box.
- Appropriate pressure values range from 0 to 5 psi, selectable in 0.1 psi increments.
- Regulator pressures can be changed during a sequencing run.

Guidelines for Setting Pressures

To set protein sequencer pressure:

1. Open the Pressures & Temperatures dialog box from the dialog box menu.



2. In the Set column, highlight the value you wish to change.
3. Enter the new value.

4. Click **Execute**.
5. To restore the original setting (if necessary), click **Revert**.

Guidelines for Setting Heater Temperatures

Follow these guidelines for setting heater temperatures. Appropriate temperature values are integer values ranging from:

- 30 °C to 70 °C for the column and cartridge heaters.
- Up to 78 °C for the flask heater.

Setting Protein Sequencer Temperatures

To set protein sequencer temperatures:

1. Open the Pressures & Temperatures dialog box from the dialog box menu.
2. Highlight the value in the Set column you wish to change.
3. Enter the new value.
4. Click **Execute**.
5. Click **Revert** to restore the original setting if necessary.

Guidelines for Activating Heaters

Follow these guidelines for activating heaters:

- Heaters are turned on and off by selecting or deselecting the appropriate option button or box.
- An On heater is on if the box is checked and the numerical value in the Set column is not grayed out.
- Only one cartridge heater at a time can be activated.
- To activate a heater, click the appropriate option button or select the check box of the On column.

Preparing the PTH-Amino Acid Standard

Use the following section to prepare the 20 Amino Acid PTH-Standard kit (P/N 400879). Skip to Preparing Final dilution when using PTH-Amino Acid Standard Solution (P/N 4340968).

Preparing a Stock Solution

WARNING **CHEMICAL HAZARD**. **R5 (0.001% DTT in acetonitrile)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: Use the R5 acetonitrile reagent for all PTH amino acid standard dilutions. This reagent contains a small amount of DTT (0.001%), which increases PTH-amino acid stability.

IMPORTANT! Read "Guidelines for Preparing Standards and Solvents" on page 2-2 before preparing the PTH-amino acid standard.

To prepare a stock solution (1 nmol of each component/10 µL):

1. Uncap each of the 3 standard vials. PTH-PE-Cys can be omitted from the standard.
2. Add 1.0 mL of R5 reagent to each vial.
3. Blanket the vials with inert gas.
4. Cap the vials and vortex thoroughly. Allow 20 min for the contents to dissolve, mixing several times during this period.
5. Store the stock solution vials at –20 °C.

Preparing a Fresh Working Solution

To prepare a fresh working solution (1 pmol each PTH-amino acid/µL):

1. Transfer 100 µL from each stock solution vial to a clean, dry 10 mL volumetric flask or graduated cylinder.
2. Add R5 reagent to bring the total volume to 10 mL.
3. Mix thoroughly.
4. Transfer the dilution to a clean, dry sequencer reagent bottle.
5. Store the working solution at –20 °C.

Preparing Final Dilution

To prepare a 10 picomole standard solution for the PROCISE instrument:

1. Add 2.5 mL of working solution or the PTH-Amino Acid Solution to a 10 mL volumetric flask.
2. Add R5 or R5B reagent to bring the volume to 10 mL.

To prepare a 1 picomole standard for the PROCISE cLC instrument:

1. Add 2.5 mL of working solution or the PTH-Amino Acid Solution to a 10 mL volumetric flask.
2. Add R5 or R5B reagent to bring the volume to 10 mL.

Storing the PTH Amino Acid Standard Solutions

Follow these guidelines for storing solutions:

- Store the stock solution at –20 °C for up to six months.
- Store the working solution at –20 °C for up to three months. The PTH-Amino Acid Standard Solution (P/N 4340968) can be stored at room temperature for one year.
- The standard can be used for peak identification on the system for one week.

Note: Several of the PTH amino acids, such as PTH-Ser, PTH-Thr, PTH-Arg, and PTH-PE-Cys, are less stable in solution than others derivatives at room temperature. Change the standard more frequently if accurate quantitation of these residues is desired.

Preparing the ß-lactoglobulin Standard

Introduction

ß-lactoglobulin (ßLG) is used as a standard for evaluating protein sequencer performance. Follow these guidelines to prepare ßLG solutions.

WARNING **CHEMICAL HAZARD**. **S4C (20% acetonitrile in water)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER **CHEMICAL HAZARD**. **R3 (trifluoroacetic acid)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! Read "Guidelines for Preparing Standards and Solvents" on page 2-2 before preparing ß-lactoglobulin standard.

Preparing the Dilution Solvent

To prepare the dilution solvent:

1. Aliquot 40 mL of S4C (10% acetonitrile/water) into a clean 2 ounce bottle.
2. Add 40 µL of R3 (trifluoroacetic acid) to the bottle and mix well.

Preparing the Stock Solution

To prepare the stock solution:

1. Add 500 µL of dilution solvent to the vial of ßLG.
2. Vortex and/or sonicate the vial to dissolve the protein. This step may require 20 min of intermittent mixing.

Note: The yield is 50 pmol/µL.

Preparing Dilutions

To prepare dilutions:

1. Rinse a clean microcentrifuge tube 3 times with the dilution solvent.
2. Dry the tube.
3. For 1 pmol ßLG/1 µL diluted solvent, add 2 µL of the stock solution and 98 µL of dilution solvent to the clean tube.
4. Gently vortex the tube until thoroughly mixed.

Storing the ß-lactoglobulin Solutions

Follow these guidelines for storing ßLG solutions:

- Store the stock solution and dilutions at –20 °C.
- Discard the stock solution after 6 months.
- Discard any dilutions of the stock solution after one week.

Changing Bottles on the Protein Sequencer

Introduction

To load fresh chemicals onto the protein sequencer, you must change bottles. The protein sequencer automatically depressurizes and backflushes the bottles to ensure operator safety during the procedure.

CAUTION **CHEMICAL HAZARD. Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: Once argon is supplied to the PROCISE Protein Sequencer, the electronic pressure system will attempt to pressurize all bottles to the settings in the Pressures & Temperatures dialog box. All bottle positions must have a bottle installed to prevent excessive argon consumption.

Removing Old Bottles

Before removing old bottles, set the protein sequencer to idle or pause.

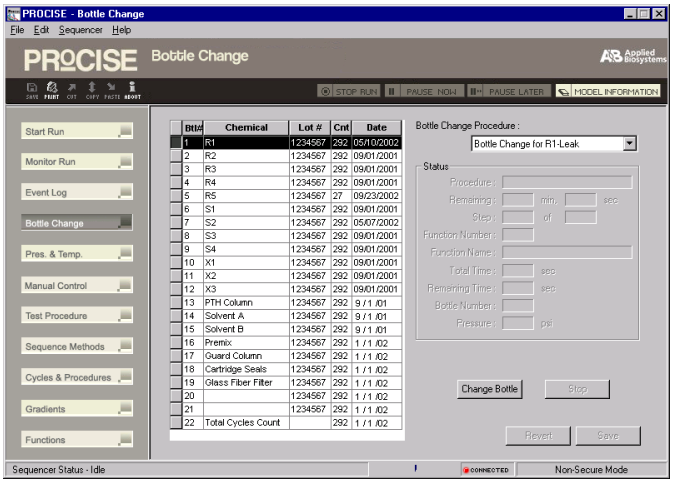
To pause a cycle:

1. Select the Pause function at the top of the screen.
2. Click **Pause Now** or **Pause Later**.

IMPORTANT! The bottle change procedures backflush old reagent back into the bottle. Do not add the contents of the old bottle to the new bottle to avoid contamination of the new bottle.

To remove the old bottle:

1. Open the Bottle Change dialog box from the dialog box menu.



Bttl#	Chemical	Lot #	Cnt	Date
1	R1	1234567	292	05/10/2002
2	R2	1234567	292	09/01/2001
3	R3	1234567	292	09/01/2001
4	R4	1234567	292	09/01/2001
5	R5	1234567	27	09/23/2002
6	S1	1234567	292	09/01/2001
7	S2	1234567	292	05/07/2002
8	S3	1234567	292	09/01/2001
9	S4	1234567	292	09/01/2001
10	X1	1234567	292	09/01/2001
11	X2	1234567	292	09/01/2001
12	X3	1234567	292	09/01/2001
13	PTH Column	1234567	292	9 / 1 /01
14	Solvent A	1234567	292	9 / 1 /01
15	Solvent B	1234567	292	9 / 1 /01
16	Premix	1234567	292	1 / 1 /02
17	Guard Column	1234567	292	1 / 1 /02
18	Cartridge Seals	1234567	292	1 / 1 /02
19	Glass Fiber Filter	1234567	292	1 / 1 /02
20		1234567	292	1 / 1 /02
21		1234567	292	1 / 1 /02
22	Total Cycles Count		292	1 / 1 /02

2. Click the bottle to be changed in the Bottle/Chemical list.
3. Choose the appropriate bottle change procedure using the Bottle Change Procedure pop-up menu.
4. Enter the lot number of the new bottle in the Lot Number window.

5. Click Change Bottle, and wait until you are prompted to remove the old bottle.
6. When prompted, remove the old bottle and bottle seal.

Installing the New Bottle

To install the new bottle:

1. Place a new seal on the rim of the new bottle.
2. Screw the new bottle into the bottle cap assembly until the bottle seal contacts the top of the assembly.
3. Tighten the bottle approximately 1/4-turn more.

   IMPORTANT! Do not tighten bottles until a snapping sound is produced by the bottle cap assembly. Ratcheting the bottle cap assembly will cause premature wear and may crack the bottle seal.
4. Click **Continue** to execute the remaining steps in the bottle change procedure.
5. Repeat the procedure steps for each additional bottle you wish to change.
6. When you are finished changing bottles, select **Save** from the File menu to save the new chemical data you entered. The main bottle change menu is then displayed.
7. If the run was paused, click **Resume** to continue the run.

Preparing the ABI 140 Pump, UV Detector, and Column for a Run

Routine operation of the ABI 140 Pump and the UV Detector is controlled by the PROCISE control software. Function 227, Prepare Pump downloads gradient programs from the computer.

Solvent gradient programming automatically changes the retention time of sample species during the course of a single chromatographic run. Both gradient programs and changes to the composition (ionic strength) of solvent A3 are used to optimize the retention times of PTH-amino acids.

The Mobile Phase

The mobile phase for this system is a controlled combination of:

- Solvents A3 and B2
- Premix Buffer Concentrate

WARNING **CHEMICAL HAZARD. A3 (3.5% tetrahydrofuran in water)** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation, central nervous system depression, and liver and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD. B2 (12% isopropanol and acetonitrile)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER **CHEMICAL HAZARD. Premix Buffer Concentrate** causes burns to the eyes, skin, and respiratory tract. It is a combustible liquid and vapor. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

The mobile phase elutes the PTH-amino acids from the column. Table 2-1 describes a typical mobile phase for this system.

Table 2-1 Typical mobile phase compositions

Chemical	PROCISE Instrument		PROCISE cLC Instrument	
	mL	P/N	mL	P/N
Solvent A3 (3.5% aqueous tetrahydrofuran/water)	1000	401464	450	401887
Solvent B2 (12% isopropyl alcohol in acetonitrile)	1000	401570	450	401886
Premix Buffer Concentrate[a]	30	401446	10	401446

Table 2-1 Typical mobile phase compositions *(continued)*

Chemical	PROCISE Instrument		PROCISE cLC Instrument	
	mL	P/N	mL	P/N
Column Temperature = 55 °C Column temperature may vary slightly for optimum separation.				

a.The amount of Premix Buffer Concentrate added must be properly adjusted to achieve optimal separation of PTH-Histidine and PTH-Arginine from other PTH-amino acids. Refer to "Positioning the Positively Charged PTH-AAs" on page 4-14 for more information.

Changing the Mobile Phase

If your system is idle for more than one week, change the mobile phase:

1. Prepare fresh solvents.
2. Optimize the separation before sequencing.

Recognizing An Aged Mobile Phase

Replace the solvents if:

- Changes in peak shape (such as broadening or tailing) occur
- An increase in baseline noise, or an unusual baseline rise occurs
- Decreased peak resolution occurs (and it cannot be corrected by minor adjustments in mobile phase composition)
- There is precipitate present in the mobile phase

Preparing Solvents A3 and B2

See "The Mobile Phase" on page 2-9 for chemical safety warnings on solvents A3, B2 and premix buffer concentrate.

WARNING **CHEMICAL HAZARD**. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! Read "Guidelines for Preparing Standards and Solvents" on page 2-2 before preparing the solvents.

To prepare solvent A3 for the PROCISE instrument:

1. Add 30 mL of Premix Buffer Concentrate to the bottle of solvent A3.
2. Invert the bottle several times to mix the contents.

   Note: Adding acetone to solvent A3 increases the UV absorbance of the solvent. This, in turn, reduces the baseline rise observed with increasing concentrations of solvent B2 during gradient elution.

3. **A**dding acetone (optional):
   a. Add a small amount of HPLC-grade 1% acetone in H_2O (approximately 1 mL) to solvent A3.
   b. Check the baseline after each addition.

4. Enter the date and lot number of the new solvent in the Bottle Change dialog box (See the figure on page 2-7) and in the protein sequencer logbook.

To prepare solvent A3 for the PROCISE cLC instrument:

1. Add 10 mL of Premix Buffer Concentrate to the bottle of solvent A3.
2. Invert the bottle several times to mix the contents.

   Note: Adding acetone to solvent A3 increases the UV absorbance of the solvent. This, in turn, reduces the baseline rise observed with increasing concentrations of solvent B2 during gradient elution.

3. **Optional** step — adding acetone:
   a. Incrementally add small amounts of HPLC-grade 1% acetone in H_2O (up to 1 mL) to solvent A3.
   b. Check the baseline after each addition.
4. Enter the date and lot number of the new solvent in the Bottle Change dialog box (See the figure on page 2-7) and in the protein sequencer logbook.

Reducing the Effects of Metal Ion Contamination

Some HPLC systems and PTH-columns exhibit symptoms of metal contamination including a negative slope in the baseline from DTT to Glu, poor resolution of certain amino acids, or low recovery of certain amino acids. Adding a small amount of phosphate ion to solvent A can alleviate these symptoms.

To prepare phosphate ion solution:

1. Prepare a 1.0 M stock solution of NaH_2PO_4 or KH_2PO_4 (monobasic sodium or potassium phosphate, sodium or potassium dihydrogen phosphate).
2. Add from 45 µL to 450 µL phosphate solution to 450 mL of solvent A3. The final concentration is 0.1–1.0 mM phosphate.

To prepare solvent B2:

1. Use solvent B2 as supplied by Applied Biosystems. No additives are required.
2. Enter the date and lot number of the new solvent in the Bottle Change dialog box (See the figure on page 2-7) and in the protein sequencer logbook.

Changing HPLC System Solvents

Changing solvents on the HPLC system involves the following major steps:

1. Purge the 140C pump.
2. Change the solvent bottles.
3. Purge the 140C pump again.

4. Run the pump to equilibrate the column.

WARNING **CHEMICAL WASTE HAZARD**. Waste Profiles in the Safety Summary describe safe handling and disposal considerations for instrument waste. Always dispose of all chemicals according to all local, federal, and state requirements. See "About Waste Profiles" on page iv-xx on page-xx for more information about the waste profile for the PROCISE C system.

Note: The following procedure for changing solvents A3 and B2 is performed through the 140C pump control panel. The keys F1, F2, F3, and F4 are referred to as *soft keys*, and are followed by the > symbol (for example, PURGE>). The prompts for which you must enter values are shown in all capitals (for example, NUMBER OF PURGES). For more information about this procedure and the 140C pump control panel, refer to the *ABI 140C Microgradient Delivery System User's Manual*.

Purging Old Solvent from the 140

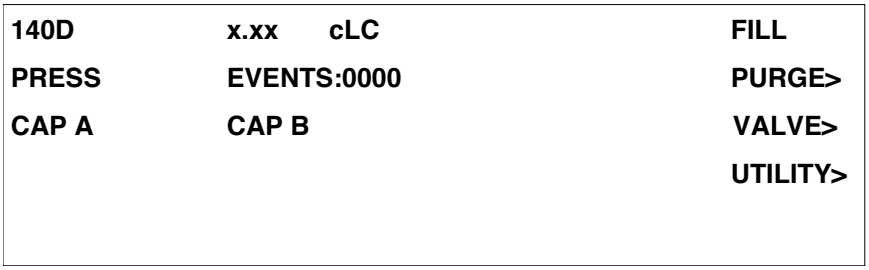
Purging old solvent is performed via the 140 pump control panel. The keys on the 140 pump control panel are referred to as *soft keys*, and are followed by the > symbol (PURGE> for example). You must enter values for the prompts that are shown in all capitals (for example, NUMBER OF PURGES).

Refer to the *ABI 140Cor D Microgradient Delivery System User's Manual* for more information on this procedure and the 140 pump control panel.

Note: Before starting this procedure, prepare fresh solvents. Instructions for preparing the solvents are on page 2-10.

To purge the old solvent from the 140 pump:

1. Remove the old solvent bottle(s).
2. Check the solvent lines for obstructions or salt deposits. If the solvent lines are not clear, clean or replace them.
3. Check all fittings for salt deposits or indications of leakage. Clean or replace as necessary.
4. From the Ready Screen (also referred to as the main menu) on the 140 pump control panel shown below, press the PURGE> soft key.



The Purge Screen is displayed.

PURGE RATE? 2,500		**BEGIN>**
SYRINGE? BOTH	**# OF PURGES? 7**	
% OF SYRINGE? 20.0	**PURGE NO.**	

5. Use the arrow keys to move the cursor to the SYRINGE prompt. Then use the Prev./Next keys to select BOTH.
6. Move the cursor to NUMBER OF PURGES, and use the numeric keypad to enter 7.
7. Move the cursor to PERCENT OF SYRINGE, and enter 20 or more. This is the percent of the syringe to empty, refill, and empty again.
8. Press the BEGIN > soft key to start the purge procedure.

Purging the 140 Pump with Fresh Solvent

To purge the 140 pump with fresh solvent:

1. To use the new solvent bottle:
   a. Place the solvent inlet line into the new bottle.
   b. Attach the cap.
   c. Place the bottle in the bottle holder.
   Repeat for each new bottle.
2. Press the BEGIN> soft key to start the purge procedure.
   The 140 pump and lines are rinsed with fresh solvent. Any air bubbles in the system are removed as well.
   The status of the procedure is displayed along the bottom of the screen on the 140 pump. To stop the purge procedure, press the Stop key.
3. Press the Manual key to enter the manual mode of operation and display the Manual Status screen. The syringes will fill with new solvent.
4. Press the FLOW> soft key. Type:
   - 40 to set the flow rate to 40 µL/min for the PROCISE cLC instrument.
   - 325 to set the flowrate to 325 µL/min for the PROCISE instrument.
5. Press the Enter key.
6. Press the %B> soft key, and type 50 to change the composition to 50% B. Then press the Enter key.
7. Press the PRESS> soft key, and type 3500 to change the maximum operating pressure to 3500 psi. Then press the Enter key.

8. Allow the 140 pump to flow at this rate and composition for 10 min to equilibrate the column.
9. Run at least 4 Flask Standard cycles to check PTH-amino acid separation efficiency and reproducibility before sequencing an unknown sample.

If the separation...	**Then...**
is essentially the same as with the old buffers	begin sequencing.
changes significantly with the new buffers	optimize the separation. **Note:** Compare and evaluate the results of the last two cycles to determine if optimization is required. If so, follow the guidelines listed under "Procise PTH-Amino Acid Separation," in Chapter 4, "Optimizing Sequencer Processes."

Preparing the PROCISE C for a Run

Sequencer Pre-Run Checklist

Table 2-2 Pre-Run Checklist

Check	Description	See Page
Pressures and Temperatures	Check the sequencer pressure and temperature settings: 1. Open the Pressures & Temperatures dialog box from the dialog box menu. The program displays the pressure and temperature settings for the instrument. 2. Using Table 2-3 on page 2-17 and Table 2-4 on page 2-17, verify the instrument pressure and temperature settings. If settings do not match the values in the tables on page 2-17, follow the appropriate procedure on page 2-17 to correct the values. 3. Activate the cartridge heaters as explained in “Activating Cartridge Heaters” on page 2-18.	2-16
Sequencer Reagents, Solvents, and Standards	Check the levels of all sequencing reagents. Replace reagents and solvents as necessary, to ensure sufficient quantities are present for the entire run. The bottle change procedure is listed in “Changing Bottles on the Sequencer” on page 2-19. **IMPORTANT!** To prevent excessive argon consumption, all bottle positions must have a bottle installed prior to pressurization. After supplying argon to the protein sequencer, the electronic pressure system pressurizes all bottles to the settings in the Pressures & Temperatures dialog box (see page 2-16).	2-18
Standard Solution(s)	Check the level and availability of the standard solutions: 1. Check the level of Alkylated Thiohydantoin- (ATH)-Amino Acid Solution sequencing standard present on the instrument (bottle C1). If additional standard is required, follow the procedures on page 2-21 to make a working stock solution. 2. If evaluating sequencer performance, follow the procedure on page 2-22 to make a working stock solution of Horse Apomyoglobin standard. **Note:** If the instrument is idle for more than one week, prepare fresh solvents and optimize the separation by running the ATH-AA standard before sequencing.	2-21
Waste Bottle	Check the fluid level of the waste bottle. If the waste level is within 2 in. of the top, empty the bottle as explained on page 2-28. **IMPORTANT!** Do not empty the waste bottle while a run is in progress.	2-28

Table 2-2 Pre-Run Checklist

Check	Description	See Page
Trap Bottle	Check the fluid level of the Trap bottle. The Trap Bottle is the polypropylene bottle mounted on the rear of the sequencer. If the Trap Bottle is 40% to 50% full, remove and empty it. Reinstall the empty bottle. **Note:** The Trap Bottle collects condensate from the waste bottle and fills very slowly.	—
Argon Cylinder	Check the pressure of the argon cylinder. If the tank pressure reading is less than 100 psi, replace the argon cylinder as explained in “Checking and Replacing the Argon Cylinder” on page 2-29.	2-29

Checking and Setting Pressures and Temperatures

Standard Pressures and Temperatures

Pressures and temperatures for the PROCISE C system are set and adjusted from the Pressures & Temperatures dialog box (Figure 2-1). If the sequencer loses pressure, or if the pressures and temperatures have been modified through functions such as the automatic leak test, click **Default** to restore the default settings.

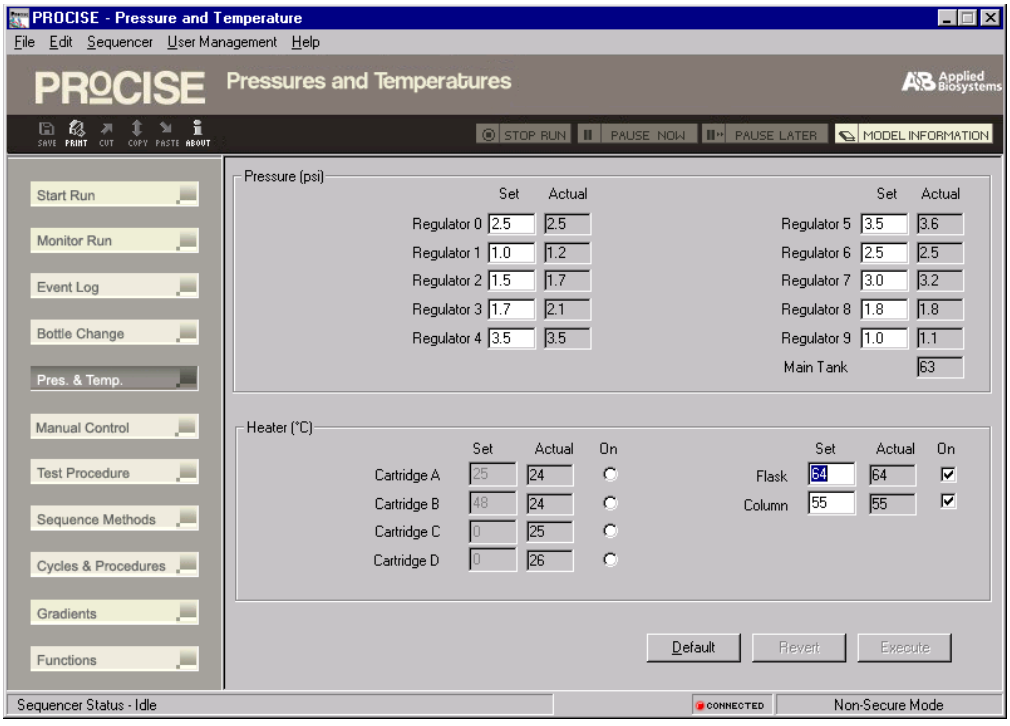


Figure 2-1 Pressures & Temperatures dialog box

Standard Pressures

Appropriate pressure values range from 0 to 5 psi, selectable in 0.1 psi increments. Table 2-3 displays the standard operating pressures for the instrument.

Table 2-3 Standard pressures for the PROCISE C protein sequencer

Regulator	C-Terminal Reagent/Function	Pressure (psi)
1	C4	2.5
2	C11	1.0
3	C3	1.5
4	C7, C8, C10	1.7
5	Cart Dry	3.5
6	C2, C9	2.5
7	C1, C5, C6	2.5
8	C12, Flask Dry	3.0
9	Flask Bubble	1.0
10	Load Injector	1.0

Standard Operating Temperatures

Appropriate temperature entries are integer values ranging from 30 °C to 70 °C for the column and cartridge heaters, and up to 78 °C for the flask heater. Table 2-4 displays the standard temperatures for instrument.

Table 2-4 Standard temperatures for the PROCISE C protein sequencer

System Component	Temperature (°C)
Cartridge	60
Flask	45
Column	45

Adjusting System Pressures

To change a pressure setting:

1. Open the Pressures & Temperatures dialog box from the dialog box menu.
2. Highlight the value in the Set column you want to change.
3. Type the new value.
4. Click **Execute**. If you make an error, click **Revert** to restore the original setting.

Adjusting System Temperatures

To change a temperature:

1. Open the Pressures & Temperatures dialog box from the dialog box menu.
2. Activate the heater by clicking the option button or selecting the check box of the On column.
3. Highlight the value in the Set column you want to change.
4. Type the new value.
5. Click **Execute**. If necessary, click **Revert** to restore the original setting.

Activating Cartridge Heaters

The PROCISE C system uses a series of cartridge heaters to drive the sequencing reaction. The instrument activates the heaters automatically before each run commences; however the system must pause first to allow the heaters to achieve the correct temperature. To avoid the pause, manually activate the heaters while preparing the instrument before the run.

You can turn heaters on and off by selecting or deselecting the appropriate check box of the *On* column. A heater is off if the option button is unchecked and the numerical value in the *Set* column is grayed out. To turn a heater on, click the appropriate option button in the *On* column. You can activate only one cartridge heater at a time.

Checking and Changing Sequencer Reagents and Solvents

Sequencer Reagents, Solvents, and Standards

Figure 2-2 illustrates the positions of the bottles on the PROCISE C protein sequencer.

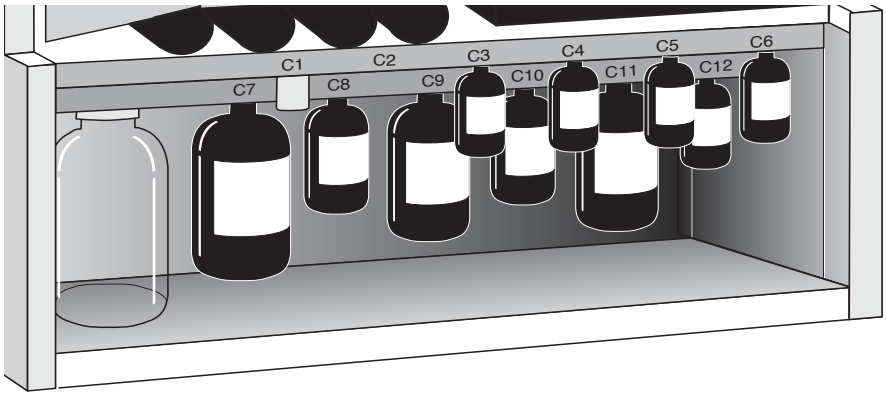


Figure 2-2 PROCISE C protein sequencer bottle configuration

All reagents and solvents supplied by Applied Biosystems are highly purified and tested to ensure optimal performance. Table 2-5 lists the reagents and solvents used for standard sequencer cycles.

Table 2-5 PROCISE C system reagents and standards

Bottle Position	Reagent/Solvent	Volume (mL)	Part Number	Storage Conditions
C1	BOC Methylnapthylthiohydantoin–Amino Acid Standard	—	403088	–20 °C[a]
C2	Empty	—	—	—
C3	N-methylimidazole/acetonitrile	40	402141	RT[b]
C4	Piperidine thiocyanate/acetonitrile	40	401700	RT[b]
C5	Acetonitrile	40	402161	RT[b]
C6	Acetic anhydride/lutidine/acetonitrile	40	402142	RT[b]
C7	Ethyl acetate	450	402160	RT[b]
C8	Bromomethylnapthalene/acetonitrile	100	401703	4 °C[a]
C9	20% acetonitrile in water	200	402162	RT[b]
C10	Tetrabutyl ammonium thiocyanate/acetonitrile	100	401854	RT[b]
C11	Diisopropylethylamine/heptane	200	401702	RT[b]
C12	Trifluoroacetic acid	50	401701	RT[b]
N/A	Horse Apomyoglobin Standard	—	402231	–20 °C[a]

a.Allow the chemicals to reach room temperature before opening them. If the bottles are opened while cold, water can condense inside them. Check the bottle caps for tightness after placing these bottles at either 4 °C (2 to 8 °C) or –20 °C (–15 to –25 °C).
b.RT (Room temperature) = 15 to 20 °C in a dark, dry place.

Changing Bottles on the Sequencer

Use the following procedure to load fresh chemicals onto the sequencer. The sequencer automatically depressurizes and backflushes the bottles to ensure operator safety during the procedure. The SequencePro™ Data Analysis Application software maintains a record of the new bottle's lot number and the date of the change.

IMPORTANT! All bottle positions must have a bottle installed to prevent excessive argon consumption. After argon is supplied to the protein sequencer, the electronic pressure system attempts to pressurize all bottles to the settings in the Pressure & Temperature dialog box.

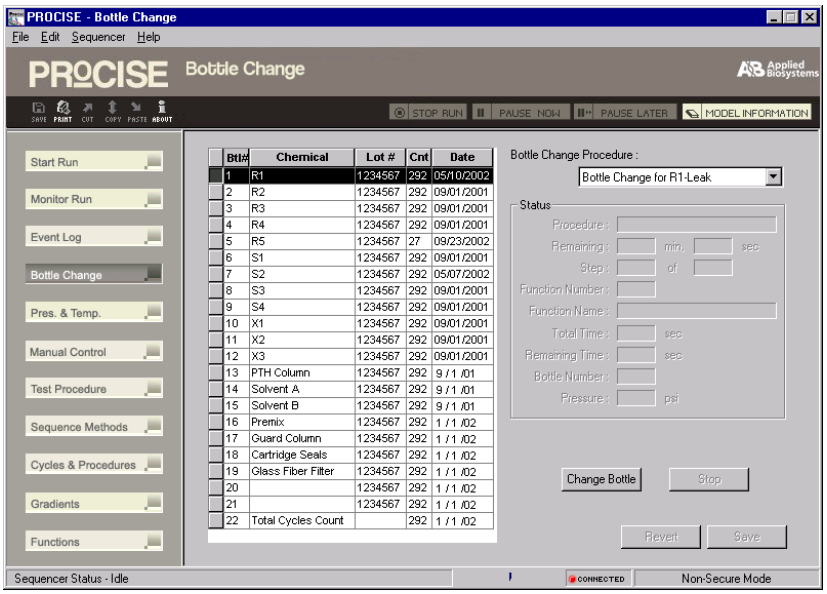
WARNING **CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous.Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

To change the bottle:

1. Select the Bottle Change dialog box from the dialog box pop-up menu.

   IMPORTANT! Do not remove the bottle at this time.

2. Select the appropriate bottle position, solvent, or column from the Bottle menu.
3. Select the appropriate procedure from the Bottle Change Procedure pop-up menu.



Btl#	Chemical	Lot #	Cnt	Date
1	R1	1234567	292	05/10/2002
2	R2	1234567	292	09/01/2001
3	R3	1234567	292	09/01/2001
4	R4	1234567	292	09/01/2001
5	R5	1234567	27	09/23/2002
6	S1	1234567	292	09/01/2001
7	S2	1234567	292	05/07/2002
8	S3	1234567	292	09/01/2001
9	S4	1234567	292	09/01/2001
10	X1	1234567	292	09/01/2001
11	X2	1234567	292	09/01/2001
12	X3	1234567	292	09/01/2001
13	PTH Column	1234567	292	9 / 1 /01
14	Solvent A	1234567	292	9 / 1 /01
15	Solvent B	1234567	292	9 / 1 /01
16	Premix	1234567	292	1 / 1 /02
17	Guard Column	1234567	292	1 / 1 /02
18	Cartridge Seals	1234567	292	1 / 1 /02
19	Glass Fiber Filter	1234567	292	1 / 1 /02
20		1234567	292	1 / 1 /02
21		1234567	292	1 / 1 /02
22	Total Cycles Count		292	1 / 1 /02

4. Configure the Bottle Change dialog box as follows:
   a. Place the cursor in the Chemical box and press the **Tab** key.
      The cursor moves to the Lot Number box, and the lot number is highlighted.
   b. Type the lot number of the new bottle into the Lot Number box.
      The SequencePro software automatically updates the Changed date.
   c. Click **Change Bottl**e.
5. When prompted, remove the old bottle and bottle seal.
6. Install the new bottle and seal:
   a. Install a new seal on the rim of the new bottle.
   b. Screw the new bottle into the bottle cap assembly, tightening it until the seal contacts the top of the bottle cap assembly.
   c. Turn the bottle approximately 1/4-turn more.

   IMPORTANT! Do not over-tighten bottles. If you hear a snapping sound (ratcheting) in the bottle cap assembly, you have tightened the bottle cap too much. Ratcheting the bottle cap assembly causes premature wear, and can crack the bottle seal.

7. Click **Continue**.

   The procedure continues through the remaining steps, which includes priming the delivery line up to the valve block.

8. Repeat the procedure steps to change additional bottles as needed.
9. When you are finished, select **Save Chemicals from the File** menu.

Preparing C-Terminal Standard Solutions

Using the Sequencer After a Lapse in Use

If the PROCISE C system is idle for more than one week, prepare fresh solvents and optimize the separation by running the ATH-AA standard before sequencing a sample.

Indicators of an aged mobile phase are:

- Changes in peak shape, such as broadening or tailing
- An increase in baseline noise, or an unusual baseline rise
- A decrease in peak resolution that you cannot correct by minor mobile phase component adjustments
- Precipitate in the mobile phase

Preparing the Alkylated Thiohydantoin (ATH)-Amino Acid Standard

WARNING **CHEMICAL HAZARD. Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: Stock solutions are 1 nmol of each component/10 µL acetonitrile.

To prepare stock solutions:

1. Uncap each vial of alkylated-amino acid (ATH-AA) standard.
2. Add 1.0 mL acetonitrile (C5 sequencer reagent).
3. Blanket each vial with inert gas.
4. Cap the vials and vortex thoroughly. Allow 20 minutes for the contents to dissolve, mixing several times during this period.
5. Store the stock solutions at 4 °C.

Working Solution

The working solution is 2.5 pmol/µL of ATH-AA standard/acetonitrile. This dilution provides a nominal 100 pmol of standard delivered to the flask during a cycle.

To prepare a fresh working solution:

1. Pipette 250 µL of each stock solution to a clean, dry 10 mL volumetric flask or graduated cylinder.
2. Add enough acetonitrile (C5) to bring the total volume to 10 mL.
3. Mix thoroughly.
4. Start the bottle change procedure for the C1 bottle position as explained on “Changing Bottles on the Sequencer” on page 2-19.
5. When prompted, remove the C1 bottle and empty any remaining standard into an appropriate waste container.
6. Rinse the bottle with a small volume of the fresh standard solution, and empty into the waste container.

7. Fill the bottle with fresh standard solution.
8. Install the bottle on the sequencer.
9. Continue with the change to purge the bottle with argon and prime the delivery line.

Note: The standard can be used for peak identification on the system for two to three weeks.

Preparing the Horse Apomyoglobin Standard Solution

Horse skeletal muscle apomyoglobin is used as a standard for evaluating sequencer performance. Use the following instructions to prepare the apomyoglobin solution.

DANGER **CHEMICAL HAZARD**. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: Stock solutions consist of 1 nmol apomyoglobin/50 µL of 0.1% trifluoroacetic acid (TFA).

To prepare the stock solution:

1. Check the label on the vial to determine the amount of horse apomyoglobin contained. Typical amounts are 73–74 nmol.
2. Add the appropriate volume of 0.1% TFA to the vial to give a final apomyoglobin concentration of 20 picomoles/µL. For example, if the vial contains 74 nmol of apomyoglobin, add 3.7 mL of 0.1% TFA.
3. Cap and vortex and/or sonicate the vial to dissolve the protein.
4. Store the stock solution at –20 °C for up to 6 months.

Preparing the HPLC System for a Run

PROCISE C System HPLC Pre-Run Check

IMPORTANT! Before sequencing a sample, be sure sufficient quantities of solvents A and B are present to complete the run. Changing HPLC solvents during sequencing can cause retention times to shift and make peak identification difficult.

The SequencePro software on the computer controls routine operation of the HPLC system (the 140C pump, the column, and the UV/VIS detector). During a sequencing run, the computer automatically downloads gradient programs to the 140C pump.

For…	See…
• a brief overview of solvent gradient programming • information about creating custom gradient programs	Chapter 5, “Custom Functions, Cycles, Methods, and Gradients.”
detailed information about the 140C pump and gradient programming **Note:** The default gradient for ATH-AA separation is in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients.”	*140C Microgradient Delivery System User’s Manual* (P/N 903078)

Using the 140C Pump After a Lapse in Use

If your system is idle for more than one week, prepare fresh solvents and optimize the separation by running the ATH-AA standard before sequencing a sample.

Indicators of an aged mobile phase are:

- Changes in peak shape, such as broadening or tailing.
- An increase in baseline noise, or an unusual baseline rise.
- A decrease in peak resolution that you cannot correct by minor mobile phase component adjustments.
- Precipitate in the mobile phase.

Preparing the HPLC Mobile Phase

Components of the Mobile Phase

The combination of solvents mixed and pumped by the 140C pump through the column and UV/VIS detector is referred to as the *mobile phase*. Changes in the relative proportion of solvents A3 and B3 during the sequencing run are stored as the gradient program. These mobile phase composition changes cause the ATH-AAs to elute from the column. Typically, the relative amount of the stronger solvent (B3) is increased over time.

Table 2-6 Mobile phase chemicals

Chemical	Quantity (mL)	Part Number
Solvent A3—3.5% tetrahydrofuran in water	1000	402254
Solvent B3—18% tetrahydrofuran in acetonitrile	1000	402255
3M Sodium Acetate Buffer, pH 3.8	25	400319
Diisopropylethylamine (DIEA)	2	400136
1% Acetone (HPLC grade)	5	—

Note: The column temperature is typically 45 °C but may vary slightly for optimum separation.

Preparing Solvents A3 and B3

IMPORTANT! Always use clean glassware when preparing solvents to minimize contaminants in the system.

WARNING **CHEMICAL HAZARD. A3 (3.5% tetrahydrofuran in water)** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation, central nervous system depression, and liver and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD. Diisopropylethylamine (DIEA)** is a flammable liquid and vapor. Exposure can cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD. 3 M sodium acetate** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD. Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare A3 solvents:

1. Open a new 1 L bottle of solvent A3.
2. Add 25 mL of 3M Sodium Acetate Buffer, pH 3.8.
3. Add 2 mL of DIEA.
4. Cap the bottle and mix well.
5. It may take a few minutes for the DIEA to dissolve.
6. Add 5.0 mL of 1.0% acetone (HPLC grade in water).

   Note: Acetone increases the UV absorbance of solvent A3 and reduces the baseline rise observed in the chromatogram during gradient elution.
7. Cap the bottle and mix well.

WARNING **CHEMICAL HAZARD. B3 (18% tetrahydrofuran in acetonitrile)** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Use solvent B3 directly as supplied by Applied Biosystems.

Changing Solvents

Changing HPLC System Solvents

Changing solvents on the HPLC system involves the following major steps:

1. Purge the 140C pump.
2. Change the solvent bottles.
3. Purge the 140C pump again.
4. Run the pump to equilibrate the column.

WARNING **CHEMICAL WASTE HAZARD**. Waste Profiles in the Safety Summary describe safe handling and disposal considerations for instrument waste. Always dispose of all chemicals according to all local, federal, and state requirements. See “About Waste Profiles” on page iv-xx on page-xx for more information about the waste profile for the PROCISE C system.

Note: The following procedure for changing solvents A3 and B2 is performed through the 140C pump control panel. The keys F1, F2, F3, and F4 are referred to as *soft keys*, and are followed by the > symbol (for example, PURGE>). The prompts for which you must enter values are shown in all capitals (for example, NUMBER OF PURGES). For more information about this procedure and the 140C pump control panel, refer to the *ABI 140C Microgradient Delivery System User’s Manual*.

Purging the 140C Pump and Changing Solvents

Purging the 140C pump rapidly expels solvents and trapped gases from the pump’s syringes. The 140C pump is equipped with an automatic purge valve to divert the flow of solvent to waste. Every time you change a solvent, equilibrate the column with the new solvents until the baseline is stable before sequencing or evaluating a separation. For additional information, refer to the *Model 140C Microgradient Delivery System User’s Manual*.

To purge the 140C pump and change solvents:

1. Remove the old solvent bottles.
2. Check the delivery system as follows:
   - Check the solvent lines for obstructions or salt deposits. If the lines are not clear, clean or replace them.
   - Check all fittings for salt deposits or indications of leakage. Clean or replace them as needed.
3. Place the solvent inlet line into the new bottle, attach the cap, and place the bottle in the bottle holder.
4. From the Ready Screen (shown below) on the 140C pump control panel, press the **PURGE**> soft key to display the Purge Screen (shown below).

   The following figure illustrates the Ready Screen:

140C	**x.xx**	**FILL>**
PRESS	**EVENTS:0000**	**PURGE>**
CAP A	**CAP B**	**VALVE>**
		UTILITY>

5. Configure the Purge Screen as follows:
   a. Use the arrow keys and numeric keypad to enter 10000 for the PURGE RATE.

      Note: This is the rate in µL/min at which the cylinders empty. The maximum value is 10000, or 10 mL/min. The smaller the value entered, the longer the purge takes.

   b. Use the arrow keys to move the cursor to the SYRINGE prompt. Then use the Prev./Next keys to enter **BOTH**.

   c. Move the cursor to NUMBER OF PURGES, and then use the numeric keypad to enter **3**.

   d. Move the cursor to PERCENT OF SYRINGE, and enter **30**.

      Note: This is the percent of the syringe to empty, refill, and empty again.

   The following figure illustrates the Purge Screen:

PURGE RATE? 10,000	**BEGIN>**
SYRINGE? BOTH	**# OF PURGES? 3**
% OF SYRINGE? 30.0	**PURGE NO.**

6. Press the **BEGIN**> soft key to start the purge procedure.

   The system rinses the 140C pump and lines with fresh solvent, removing any air bubbles in the system.

   Note: The status of the procedure is displayed along the bottom of the screen on the 140C pump. To stop a purge procedure, press the Stop key.

Equilibrating the Column

To equilibrate the column with fresh solvent:

1. Purge the 140C pump again three times at 30% to thoroughly rinse the tubing and cylinders with fresh solvent and to remove air bubbles from the system.
2. Press the Manual key to enter the manual mode of operation and display the Manual Status Screen (see below).

TIME	**FLOW**	**%B**	**FLOW>**
PRESS	**EVENTS:0000**		**%B>**
CAP A	**CAP B**		**PRESS>**
			REFILL>

The syringes fill with new solvent.

3. Change the flow rate to 300 µL/min:
   a. Press the FLOW> soft key.
   b. Type **300**.
   c. Then press the **Enter** key.
4. Change the composition to 50 %B:
   a. Press the **%B**> soft key.
   b. Type 50.
   c. Press the **Enter** key.
5. Change the maximum operating pressure to 3500 psi:
   a. Press the **PRESS**> soft key.
   b. Type **3500**.
   c. Press the **Enter** key.

   Allow the 140C pump to operate at this flow rate and solvent composition for 5 min.
6. Run at least 2 C-term Flask Standard cycles to check ATH-amino acid separation efficiency and reproducibility before sequencing an unknown sample.

Emptying the Waste Bottle

Emptying the Waste Bottle on the Instrument

Empty the waste bottle when the waste level is within 2 in. of the top. Do not empty the waste bottle while a run is in progress.

WARNING **CHEMICAL WASTE HAZARD**. Waste produced by the sequencer can be hazardous and can cause injury, illness, or death. Only operate a vented instrument if it is connected in accordance with all the requirements. Handle all liquid, solid, and gaseous waste as potentially hazardous. Sequencer waste must be disposed of properly and carefully in accordance with all state, local, and federal requirements. Refer to the Waste Profile in the *PROCISE and PROCISE cLC Protein Sequencing System Site Preparation and Safety Guide* or the *PROCISE C Protein Sequencing System Site Preparation and Safety Guide* for proper disposal of sequencer waste. When handling the waste for disposal, wear gloves and use eye protection. Avoid inhalation and skin contact.

To empty the waste bottle:

1. Raise the black bar above the waste bottle, so that the cap assembly disengages fully from the top of the bottle.
2. Carefully pull the bottle out, keeping the bottle level at all times.

   IMPORTANT! Immediately cover the bottle to contain the vapors.
3. Discard the waste as described in the Waste Profile in the *Procise Protein Sequencing System Site Preparation and Safety Guide*.
4. Raise the black bar, reinstall the waste bottle, and release the bar.
5. Inspect the top of the bottle where it seals against the O-ring on the waste manifold. The entire o-ring seal should be inside the bottle and flattened against the bottle surface.

CAUTION Besides collecting waste, the waste bottle assists venting by acting as a low pressure area. Chemical deliveries flow from high pressure (reagent or solvent bottles) to low pressure (vent or waste). Therefore, for flow to occur, the waste bottle and its associated delivery and exhaust lines must be open to normal atmospheric pressure or reduced pressure (as in a fume hood) and not restricted in any way. If the waste bottle is not effectively vented, gas and liquid deliveries are impeded.

Checking and Replacing the Argon Cylinder

Purpose of the Argon Gas

Argon is used to pressurize bottles, move fluids, and dry flow paths within the protein sequencer. Argon is usually supplied from a compressed gas cylinder placed near the sequencer.

IMPORTANT! Replace the argon cylinder when the tank pressure falls below 100 psi.

CAUTION **CHEMICAL HAZARD**. **Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Materials Required

The following materials are required to replace the argon cylinder:

- Large wrench (for removing the argon tank regulator)
- Argon cylinder valve key (if necessary)
- Cylinder blanking plug removal tool (if necessary)
- Teflon™ tape (if necessary)
- Safety goggles

WARNING **EXPLOSION HAZARD**. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets, chains, or clamps.

Removing the Argon Cylinder

To remove the old cylinder:

1. Note the current operating pressure on the regulator.
2. Turn off the argon tank shutoff valve.
3. Open the purge valve on the rear of the instrument to bleed off any residual pressure.
4. Remove the regulator and carefully set it down.
5. Remove the old cylinder.

Installing a New Argon Cylinder

To install the new cylinder:

1. Fasten the new cylinder securely in place.
2. Wearing safety goggles, briefly open and close the cylinder shutoff valve to remove any debris that might have settled.
3. Screw the regulator onto the new cylinder. If appropriate, use Teflon tape.
4. Open the cylinder shutoff valve.

   Gas starts to flow out of the instrument purge valve.
5. Close the tank regulator by turning the adjustment knob fully counter clockwise.

Performing a Leak Test of the Argon Cylinder

To leak test the new cylinder:

1. Close the tank shutoff valve and wait 30 sec.
2. Using tape, mark where the needle is registering on the tank high-pressure regulator gauge.
3. Wait 1 min and note the reading on the high-pressure gauge.

Did the pressure drop?	Then…
Yes	the sequencer is leaking. Determine the cause of the leak, repair it, and repeat this procedure.
No	there are no leaks in the system. Turn on the tank shutoff valve.

4. Adjust the tank regulator to the recommended operating pressure of 65 psi.

Preparing and Running Samples 3

This chapter describes the sample preparation, sample loading, and starting a run on the PROCISE® Protein Sequencer Systems.

The following topics are contained in this chapter:

Preparing Samples for N-Terminal Sequencing

Modifying Cystines and Cysteines (Optional)

Chemicals Required

Chemical	Part number
Guanidine hydrochloride (Gu-HCl), 99%	(Aldrich) 17,725-3
Tris(hydroxymethyl)aminomethane (TRIS), 99.9+%, ultrapure grade	(Aldrich) 15,456-3
Dithiothreitol (DTT), 98%	(Aldrich) 15,046-0
Acrylamide, 99+%, electrophoresis grade	(Aldrich) 14,866-0
Hydrochloric acid (HCl), reagent grade	—
Water, deionized or HPLC grade (DI water)	—

Solutions Required

Solution	Procedure
6 M Gu-HCl, 0.3 M TRIS, pH 8.5	1. Using volumetric glassware, add 57.3 g Gu-HCl, 3.63 g TRIS and water to a final volume of approximately 90 mL water. 2. Stir or mix to dissolve. 3. Adjust the pH to 8.5 with HCl. 4. Bring the final volume to 100 mL by adding DI water. 5. Store the final solution at room temperature after filtering it through a 0.22 µm sterilization filter.
30% (w/v) acrylamide/ water, 4.22 M	Dissolve 3 g of acrylamide in water for every 10 mL final volume.
10 mM DTT	Prepare fresh by dissolving 1.5 mg of dithiothreitol in 1.0 mL water.

WARNING **CHEMICAL HAZARD**. **Guanidine hydrochloride** may be harmful if swallowed or absorbed through the skin. Exposure may cause eye, skin, and respiratory tract irritation and adverse effects on the central nervous system and bone marrow. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD**. **Dithiothrietol (DTT)** may cause eye, skin, and respiratory tract irritation, central nervous system depression, and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **Polyacrylamide** may contain residual amounts of acrylamide monomer. Acrylamide is harmful if in contact with the skin or if swallowed. Acrylamide may cause eye, skin, and respiratory tract irritation. It may also cause an allergic reaction. Exposure may cause damage to the nervous system, kidneys, and reproductive system. Acrylamide is a possible cancer and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Modification Procedure

To modify cystines and cysteines (Cys) for 100 pmoles-5 nmoles of protein:

1. Denature the protein by dissolving or diluting the protein solution into 6 M Gu-HCl solution.

   Ensure that the final concentration of Gu-HCl exceeds 4 M and that the final volume is 100 to 400 µL.

2. Reduce the disulfide bonds by adding 10 mM DTT solution so the molar ratio of DTT to disulfide bridges is approximately 100:1.

   For example, a 1 nmol sample of lysozyme (8 cysteine residues, or 4 disulfide bridges) requires the addition of 400 nmol DTT, or 40 µL of 10 mM DTT.

3. Incubate the solution, preferably at 55 to 60 °C for 30 to 60 minutes, or for 4 hours at room temperature.

4. Alkylate the cysteines by adding 30% acrylamide solution (4.22 M) so the molar ratio of acrylamide to DTT is approximately 100:1.

   Based on the example in Step 2 above, approximately 40 µmol acrylamide, or approximately 10 µL of acrylamide solution, is required.

5. Incubate the solution, preferably at 55 to 60 °C for 60 minutes, or overnight at room temperature.

6. Apply the alkylated sample to the PVDF membrane in a ProSorb Sample Preparation Cartridge using the procedure “Sample Application to a PVDF Membrane” on page 3-5.

Applying the Protein Sample to a PVDF Membrane

Chemicals Required

Chemical	Part Number
Methanol, HPLC grade	—
Water, deionized or HPLC grade (DI water)	—
Trifluoroacetic acid (TFA), protein sequencing grade, 40 mL	400003

Materials Required

Material	Part Number
ProSorb Starter Kit, includes: • ProSorb cartridges (10 pack) • ProSorb filters refill pack (50) • Membrane Removal Punch (1)	402139
ProSorb cartridge, (10 pack)[a]	401950
ProSorb inserts, Refill Pack (25)	402052
ProSorb filters, Refill Pack (50)	402050
Filtration equipment and 0.22 µm sterilization filters (aqueous)	—

a.General instructions on the use of ProSorb Sample Preparation Cartridges are provided below.

Solutions Required

Solution	Procedure
6 M Gu-HCl, 0.3 M TRIS, pH 8.5	See “Solutions Required” on page 3-2 for an explanation of how to prepare this solution.
2.0% (v/v) TFA/DI water	Add 0.2 mL trifluoroacetic acid to 10 mL DI water.
0.1% (v/v) TFA/DI water	Add 0.1 mL trifluoroacetic acid to 100 mL DI water.

WARNING **CHEMICAL HAZARD**. **Guanidine hydrochloride** may be harmful if swallowed or absorbed through the skin. Exposure may cause eye, skin, and respiratory tract irritation and adverse effects on the central nervous system and bone marrow. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER **CHEMICAL HAZARD**. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Sample Application to a PVDF Membrane

Note: If you reduced and alkylated your sample (see "Modifying Cystines and Cysteines (Optional)" on page 3-2), skip steps 1 and 2 and continue to step 3.

To apply your sample to a ProSorb PVDF membrane:

1. Prepare a clean workspace.
2. Dissolve or dilute the protein solution using either 2% TFA or 6 M Gu-HCl solution. The diluent must be at least 50% of total volume.

   The final volume must be less than 400 µL.
3. Assemble the appropriate number of filters and inserts.
4. Wet the PVDF membrane in each ProSorb cartridge with 15 µL of methanol.
5. Press the insert so that it fits snugly against the filter.
6. Add 50 µL of 0.1% TFA into each insert.

   Allow all the fluid to be drawn through the membrane.
7. Add the protein solutions into the inserts.

   Allow all the fluid to be drawn through the membrane.
8. Choose from the following:

If you loaded the sample in…	Then…
6 M Gu-HCl	wash the membrane with 50 µL of 0.1% TFA.
aqueous TFA	it is not necessary to wash the membrane.

9. Remove the insert from the ProSorb cartridge, and dry at 55 to 60°C for at least 10 min.

   IMPORTANT! Protect the PVDF surface from dust or other contamination.

   IMPORTANT! The PVDF membrane must be completely dry before proceeding to "Procise C Only – Derivatize with Phenyl Isocyanate." Any water present on the membrane will hydrolyze the phenyl isocyanate.

Sequencing Liquid Samples

Two sequencing methods are available for liquid samples:

- Pulsed-liquid
- Gas-phase

Sequencing Methods for Liquid Samples

Table 3-1 Sequencing methods for liquid samples

Method Name	Description
Pulsed-liquid	• Delivers an small aliquot of liquid TFA to the cartridge for cleavage after coupling. • Offers slightly higher repetitive yields than the Gas-phase method.
Gas-phase	• Delivers TFA vapor for the cleavage. • Offers lower background than the Pulsed-liquid method. • May require optimization to sequence samples on glass- fiber filters. Refer to page 3-6 for more information.

Precycling Glass-Fiber Filters

Before loading your sample onto a glass-fiber filter, you must:

- Apply BioBrene™ Plus to the filter
- Precycle the filter using the Filter Precycle method

Note: Precycling is necessary because the BioBrene solution may contain small amounts of compounds that could interfere with sequencing. The Filter Precycle method washes and conditions the BioBrene-coated filter by running several short cycles of Edman chemistry.

Sequencing Blotted/Membrane-bound Samples

Two Methods

Two methods are available for sequencing blotted samples:

- Pulsed-liquid PVDF
- Gas-phase PVDF

Table 3-2 Blotted sample sequencing methods

Method Name	Description
Pulsed-liquid PVDF	• Delivers an aliquot of liquid TFA to the cartridge for cleavage after coupling. • Offers slightly higher repetitive yields than the Gas-phase PVDF method.
Gas-phase PVDF	• Delivers TFA vapor for the cleavage. • Has a 45 min cycle time. • Offers lower background than the Pulsed-liquid PVDF method.

Preparing Protein Samples for C-Terminal Sequencing

Procedure Description

The following instructions describe how to prepare liquid protein samples for C-terminal sequencing. To prepare electroblotted samples for C-terminal sequencing, only step 3 is required.

Three preparation steps are involved:

Step	Description	Page
1.	Modify the cystines and cysteines in your sample (optional). Modifying the cystines and cysteines (Cys) in your sample is an optional step. Unmodified cysteine residues yield only a small amount of dehydroalanine upon sequencing making it impossible to distinguish cysteine residues from serine (Ser) residues, which also yield dehydroalanine. Reduction and alkylation of the protein yields a derivative of cysteines with a unique retention time, resulting in unambiguous identification of cysteine residues.	3-2
2.	Apply the protein sample to a PVDF membrane. Prior to being loaded onto the sequencer, all samples must be applied to a solid sequencing support. Polyvinylidene difluoride (PVDF) membrane is an ideal support for use with C-terminal sequencing chemistry. The ProSorb® Sample Preparation Cartridge simplifies the process of reliably applying protein to the PVDF membrane.	3-4
3.	Derivatize the sample with phenyl isocyanate. After the sample has been applied to the membrane and dried, phenyl isocyanate derivatizes the ε-amino group of lysine. This process provides a stable, identifiable derivative of any lysine residue. This step also increases sample hydrophobicity, thus increasing the sample's affinity for the membrane.	3-8

Procise C Only – Derivatize with Phenyl Isocyanate

Chemicals Required

Chemical	Part number
Diisopropylethylamine (DIEA), Peptide synthesis grade, 175 mL	400136
Anhydrous acetonitrile (ACN), DNA synthesis grade, 30 mL	400060
Phenyl isocyanate (PIC), Reagent Grade	—

Materials Required

Material	Part number
PVDF Membrane Removal Punch	401397

Solutions Required

Solution	Procedure
DIEA/ACN solution	Add 660 µL of DIEA into a 30 mL bottle of anhydrous acetonitrile, resulting in a 0.124 M DIEA solution.
PIC/ACN solution	Add 660 µL of phenyl isocyanate into a 30 mL bottle of anhydrous acetonitrile, resulting in a 0.198 M PIC solution.

WARNING **CHEMICAL HAZARD**. **Diisopropylethylamine (DIEA)** is a flammable liquid and vapor. Exposure can cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER **CHEMICAL HAZARD. Phenyl isocyanate** causes burns to the eyes, skin, and respiratory tract. It may be harmful if inhaled, swallowed, or absorbed through the skin. Exposure may cause allergic skin and respiratory reactions. It is a combustible liquid and vapor. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Derivatization Procedure

IMPORTANT! The PVDF membrane must be completely dry before starting the derivatization procedure. Any water present on the membrane will hydrolyze the phenyl isocyanate.

To derivatize the sample with phenyl isocyanate:

1. Wet the PVDF membrane with 3 µL of PIC solution.
2. Using a clean pipette tip, wet the membrane with 3 µL of DIEA solution.
3. Incubate at 55 to 60 °C for 5 to 10 min. The sample is now ready for sequencing.

Loading Samples

BioBrene Plus Storage, Preparation, and Use

Overview

BioBrene Plus is a cationic polymer that improves the binding of proteins and especially peptides to glass fiber or PVDF membrane. Its use provides a significant improvement in sequencing efficiency (repetitive yield).

Guidelines

Follow these guidelines for the use of BioBrene Plus:

- We strongly recommend storing small volumes of the reconstituted BioBrene (20 µL each) in individual Eppendorf tubes.
- Always use fresh BioBrene when preparing a methanolic dilution; otherwise, sequencing yields may be reduced due to solution degradation.
- For best results, use methanolic dilutions within 48 hours after preparation or thawing.

CAUTION **CHEMICAL HAZARD**. **Biobrene Plus** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER **CHEMICAL HAZARD**. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing BioBrene Plus Stock Solution

To prepare BioBrene Plus stock solution for GFC filters:

1. Follow the instructions enclosed with the BioBrene Plus (P/N 400385) to prepare your stock solution.
2. Divide the stock solution into one-time use amounts by aliquotting 20 µL of BioBrene into individual Eppendorf tubes.
3. Freeze the tubes of BioBrene.

Preparing Dilutions for PVDF Membrane-bound Samples

To prepare BioBrene Plus dilutions for PVDF samples:

1. Thaw one of the tubes containing 20 µL of reconstituted BioBrene.
2. Add 70 µL methanol, and 10 µL 0.1% TFA to the BioBrene (100 µg/µL).
3. Vortex the solution for 5 sec.

Sample Loading Overview

The following pages describe how to load various sample types onto the reaction cartridges (Figure 3-1 on page 3-11). Separate loading instructions are provided for:

- Samples prepared in ProSorb sample preparation cartridges
- Electroblotted samples
- Liquid samples

Materials Required to Load Samples onto the Protein Sequencer

- PROCISE cartridge seals (P/N 401950)
- BioBrene solution
- ProSorb membrane punch tool (if using ProSorb Sample Preparation cartridges; P/N 401397)
- Self-closing forceps

Note: Instructions for preparing BioBrene solution are provided on page 3-9.

Loading Samples Prepared in ProSorb Cartridges

Removal, Disassembly and Cleaning of the Reaction Cartridge

IMPORTANT! Sample and cartridge contamination must be minimized to ensure optimal sequencing results.

- Always wear gloves and use forceps when handling seals and sample supports.
- All forceps, pipette tips, glassware and other hardware used should be clean and dedicated for sample preparation.

HOT COMPONENTS. Some components on the sequencer may be hot! Use caution when working with hot components to avoid injury.

To remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 3-1 on page 3-11).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Discard the used PROCISE cartridge seal and sample support from the previous run.
6. Clean the upper and lower glass cartridge blocks by rinsing the inner surfaces of both blocks with methanol.
7. Place each block in the cartridge block drying assembly on the sequencer, and dry them with a stream of argon.
8. Referring to Figure 3-2 on page 3-11, place the Teflon™ seal back into the cartridge block holder if it came out during disassembly.

Diagrams

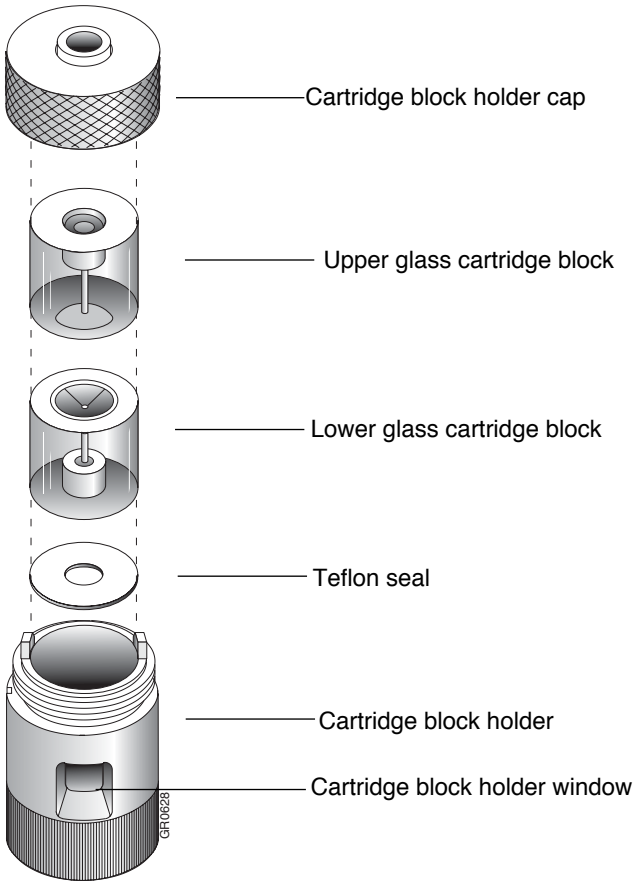


Figure 3-1 6 mm reaction cartridge components in upright position

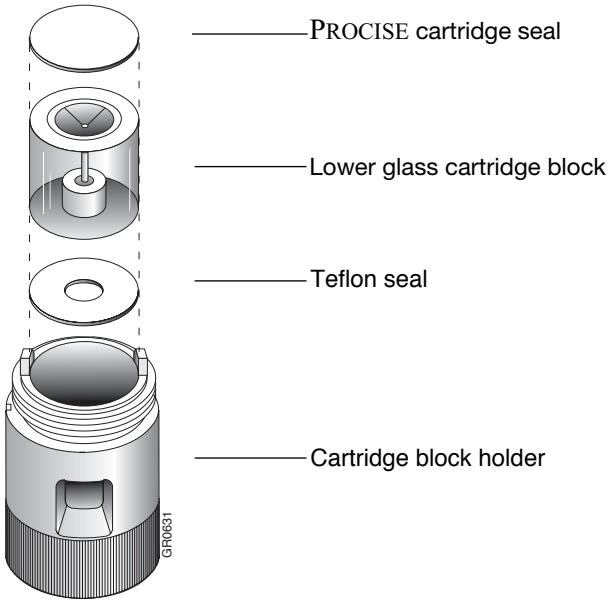


Figure 3-2 Reassembling the lower portion of the reaction cartridge

Loading the Sample

To load the sample:

1. Insert the lower glass cartridge block into the cartridge block holder.
2. Place the upper glass cartridge block, sample wells facing up, on a clean, dry surface.
3. If you have not already punched the PVDF membrane into the ProSorb sample reservoir insert, remove the insert from the holder now.

   If you have already punched the membrane into the insert, proceed to step 4.

   IMPORTANT! Do not allow the membrane to touch anything except the ProSorb insert, the forceps, and the glass cartridge block.
4. Discard the filter, and set the holder aside for cleaning and reuse.
5. Keeping the cap closed, carefully push the PVDF membrane into the sample reservoir insert using the punch tool (Figure 3-3).
6. Using self-closing forceps, remove the membrane from the ProSorb insert and place in the sample well of the upper glass cartridge block.
7. Apply 3µL of diluted BioBrene Plus solution to the membrane, cover with a new cartridge seal and allow it to dry. Instructions for preparing this solution are on page 3-9.
8. Using forceps, place the cartridge seal on top of the lower glass cartridge block.

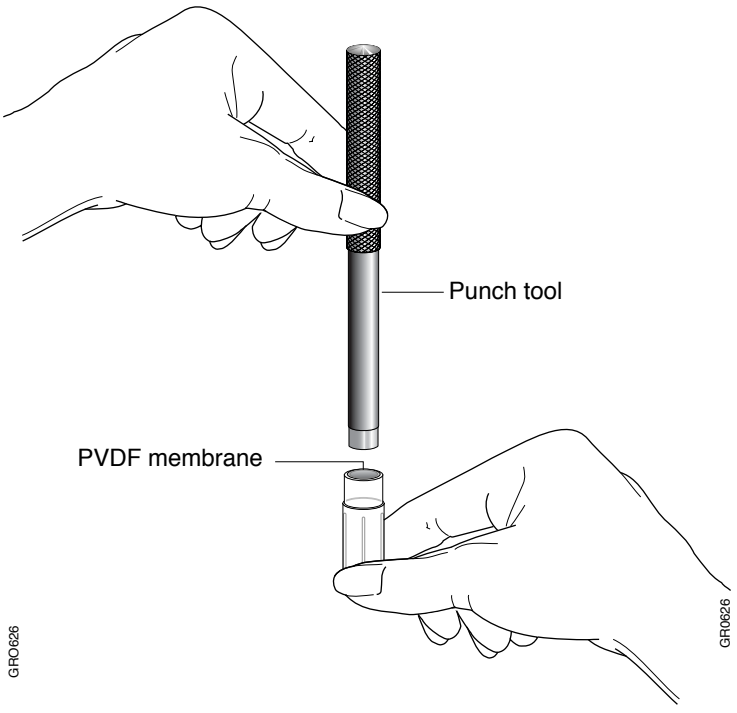


Figure 3-3 Punch the PVDF membrane into the ProSorb sample reservoir insert

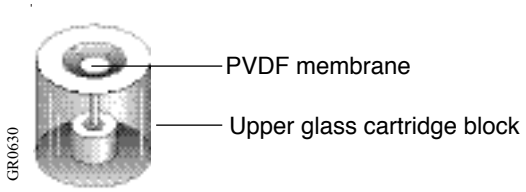


Figure 3-4 PVDF membrane centered in well of upper glass cartridge block

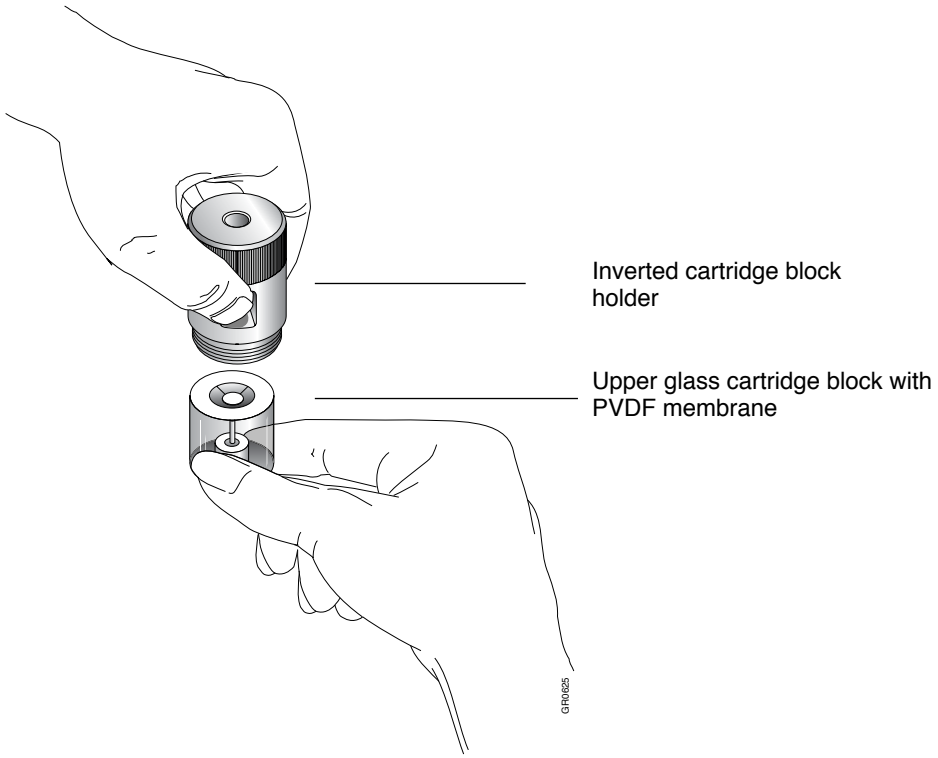


Figure 3-5 Slide the upper glass cartridge block with PVDF membrane up into the cartridge block holder

Reassembly and Leak Testing the Reaction Cartridge

To reassemble and leak test the reaction cartridge(s):

1. Hold the lower glass cartridge block and PROCISE cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 3-5.
2. Center the membrane in the sample well and slide the upper glass cartridge block with the sample up into the cartridge block holder until it is flush against the lower cartridge block.
3. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.
4. Place the reaction cartridge back into the cartridge assembly on the sequencer.

5. Reassemble the cartridge assembly:
   a. Insert the completed block holder in the cartridge assembly.
   b. Place the cap on top of the assembly, align the guide pins and slots, then press down and turn the cap until it stops against the body of the assembly.

   Note: The seal between the cartridge blocks and the Kel-F ferrules is made by spring force. Overtightening the cartridge assembly does not increase the sealing force.
6. Perform a cartridge leak test by following the instructions below.

You are now ready to sequence your sample. Turn to page 3-24 for instructions on starting a run.

Performing a Cartridge Leak Test

A cartridge leak test should be performed prior to every run to verify that the cartridge assembly is leak tight.

During the test:

- The cartridge is pressurized to 3.5 psi
- The pressure drop is monitored for 20 sec.

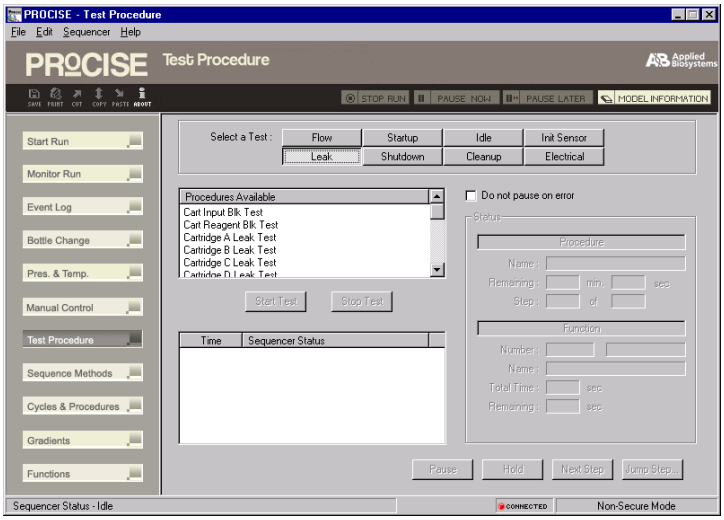
Test results are reported in the event log at the end of the test.

Leak Test Procedure

Note: The protein sequencer must be idle to perform a leak test.

To start the leak test:

1. Select the Test dialog box from the dialog box pop-up menu.



2. Click **Leak**.

3. Scroll through the test menu, and select the cartridge(s) to be tested. Hold down the Command or shift key to select more than one cartridge.
4. Click **Start Test**.

IMPORTANT! Interrupting this procedure can invalidate the test results. In addition, the pressure regulator may not be reset to the correct pressure. User intervention commands, such as Jump Step and Pause, should never be used during a leak test.

If a Reaction Cartridge Fails a Leak Test

When a reaction cartridge fails a leak test, perform the three procedures below:

To remove and disassemble the reaction cartridge:

1. Remove the reagent inlet cap.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 3-1 on page 3-11).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.

To inspect the sample matrix and cartridge seal:

1. Check the position of the sample. Is it centered in the well of the upper glass cartridge block? If not, recenter the sample.
2. Check the PROCISE cartridge seal for tears or unevenness in the sealing impression. Even if the seal appears correct, discard the seal and insert a new seal.

To reassemble and leak test the reaction cartridge:

1. Reassemble the reaction cartridge.
2. Check the Kel-F ferrules on the reagent inlet cap for damage or foreign materials. Repair or clean the cap if necessary.
3. Place the reaction cartridge into the cartridge assembly on the protein sequencer.
4. Reassemble the cartridge assembly:
   a. Insert the completed block holder in the cartridge assembly.
   b. Place the cap on top of the assembly, align the guide pins and slots, then press down and turn the cap until it stops against the body of the assembly.

   Note: The seal between the cartridge blocks and the Kel-F ferrules is made by spring force. Overtightening the cartridge assembly does not increase the sealing force.
5. Repeat the cartridge leak test.

Loading Electroblotted Samples

Before Loading

We strongly recommend you apply a small amount of BioBrene solution to the sample before loading your sample onto the reaction cartridge. Instructions for preparing this solution are on page 3-9.

Removal, Disassembly and Cleaning of the Reaction Cartridge

IMPORTANT! Always wear gloves and use forceps when handling seals and sample supports.

- All forceps, pipette tips, glassware and other hardware used should be clean and dedicated for sample preparation.
- Sample and cartridge contamination must be minimized to ensure the best sequencing results.

WARNING **HOT COMPONENTS.** Some components on the sequencer may be hot! Use caution when working around hot components to avoid injury.

WARNING **CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD**. **Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 3-1 on page 3-11).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Discard the used PROCISE cartridge seal and sample support from the previous run.
6. Clean the upper and lower glass cartridge blocks by rinsing the inner surface of both blocks with methanol.
7. Place each block in the cartridge block drying assembly on the sequencer, and dry them with a stream of argon.
8. Referring to Figure 3-2 on page 3-11, place the Teflon seal back into the cartridge block holder if it came out during disassembly.
9. Insert the lower glass cartridge block into the cartridge block holder.

Loading the Sample

To load the sample:

1. Insert the lower glass cartridge block into the cartridge block holder.
2. Place the upper glass cartridge block, sample wells facing up, on a clean, dry surface.
3. If you have not already punched the PVDF membrane into the ProSorb sample reservoir insert, remove the insert from the holder now.

   If you have already punched the membrane into the insert, proceed to step 4.

   IMPORTANT! Do not allow the membrane to touch anything except the ProSorb insert, the forceps, and the glass cartridge block.
4. Using self-closing forceps, remove the membrane from the ProSorb insert and place in the sample well of the upper glass cartridge block.
5. Apply 3µL of diluted BioBrene Plus solution to the membrane, cover with a new cartridge seal and allow it to dry. Instructions for preparing this solution are on page 3-9.
6. Using forceps, place the cartridge seal on top of the lower glass cartridge block.

Reassembling and Leak Testing the Cartridge

To reassemble and leak test the reaction cartridge(s):

1. Hold the lower glass cartridge block and PROCISE cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 3-5 on page 3-13.
2. Slide the upper glass cartridge block with the sample up into the cartridge block holder until it is flush against the lower cartridge block.
3. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.
4. Place the reaction cartridge into the cartridge assembly on the sequencer.
5. Reassemble the cartridge assembly:
   a. Insert the completed block holder in the cartridge assembly.
   b. Place the cap on top of the assembly, align the guide pins and slots, then press down and turn the cap until it stops against the body of the assembly.

   Note: The seal between the cartridge blocks and the Kel-F ferrules is made by spring force. Overtightening the cartridge assembly does not increase the sealing force.
6. Perform a cartridge leak test by following the instructions on page 3-14.

You are now ready to sequence your sample. Turn to page 3-24 for instructions on starting a run.

Loading Liquid Samples onto Glass-Fiber Filters

IMPORTANT! Always wear gloves and use forceps when handling seals and sample supports.

- All forceps, pipette tips, glassware and other hardware used should be clean and dedicated for sample preparation.
- Sample and cartridge contamination must be minimized to ensure the best sequencing results.

WARNING **HOT COMPONENTS.** Some components on the sequencer may be hot! Use caution when working around hot components to avoid injury.

WARNING **CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD**. **Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD**. **Biobrene Plus** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Step 1 — Load and Treat the Glass-fiber Filter

To remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 3-1 on page 3-11).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Discard the used PROCISE cartridge seal and sample support from the previous run.
6. Clean the upper and lower glass cartridge blocks by rinsing the inner surface of both blocks with methanol.
7. Place each block in the cartridge block drying assembly on the sequencer, and dry them with a stream of argon.

To load and treat a glass-fiber filter:

1. Referring to Figure 3-2 on page 3-11, place the Teflon seal back into the cartridge block holder if it came out during disassembly.
2. Insert the lower glass cartridge block in the cartridge block holder.
3. Using forceps, place a new cartridge seal on top of the lower glass cartridge block.
4. Center a new glass-fiber filter in the well of the upper glass cartridge block (Figure 3-4 on page 3-13).
5. Gently press the filter in place with the tamper tool.

   Note: An off-center filter can cause cartridge sealing problems. Rips or holes in the filter will reduce sequencing efficiency.
6. Load BioBrene solution onto the center of the filter. Use:
   - 7.5 µl for PROCISE cLC instrument
   - 15 µL for the PROCISE instrument

   Instructions for preparing this solution are on page 3-9.

   Note: The volume of BioBrene solution applied to the filter must be sufficient to wet the entire filter. Additional fluid can be loaded if the filter is dried between loadings.
7. To dry the filter, place the upper cartridge block in the cartridge drying assembly with the filter facing up.
8. Lower the drying arm. Argon will flow to dry the filter automatically for 5 min.
9. If not completely dry, raise and lower the drying arm again for an additional 5 min.

To reassemble and leak test the reaction cartridge(s):

1. Hold the lower glass cartridge block and PROCISE cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 3-5 on page 3-13.
2. Slide the upper glass cartridge block up into the cartridge block holder until it is flush against the lower cartridge block.
3. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.
4. Place the reaction cartridge into the cartridge assembly on the sequencer.
5. Reassemble the cartridge assembly:
   a. Insert the completed block holder in the cartridge assembly.
   b. Place the cap on top of the assembly, align the guide pins and slots, then press down and turn the cap until it stops against the body of the assembly.

Note: The seal between the cartridge blocks and the Kel-F ferrules is made by spring force. Overtightening the cartridge assembly does not increase the sealing force.

Step 2 — Perform a Cartridge Leak Test

Perform a cartridge leak test by following the instructions on page 3-14.

Step 3 — Precycle the Glass Fiber Filter

Precycling the glass fiber filter takes 2.5 h.

To precycle the glass-fiber filter:

1. Select the Start Run dialog box from the dialog box pop-up menu on the computer (Figure 3-6).
2. Set the run order of your cartridges.
3. Enter a unique file name for each cartridge.
4. Enter 5 for the number of cycles.
5. If additional BioBrene is used, more cycles are required. For example, at least 6 cycles are required to precycle a filter loaded with 15 µL of BioBrene.
6. Select **Filter Precycle** for the method.
7. Select **Collect Data**.
8. Click **Start Run**.

The filter precycle procedure will take approximately 2.5 hours. When complete, the status line on your monitor will read “idle”. You are now ready to load your sample.

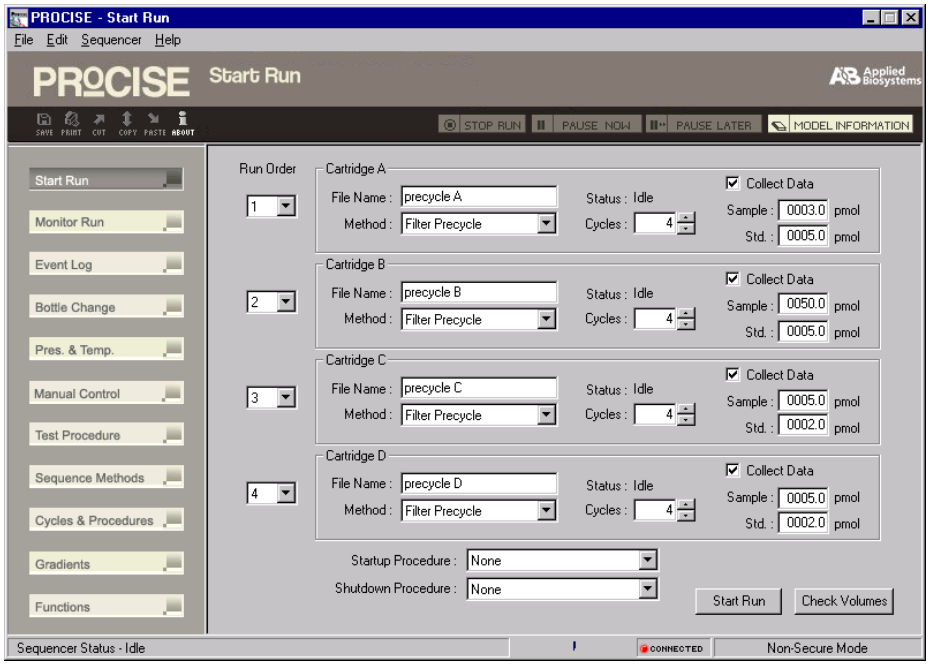


Figure 3-6 Precycling a glass-fiber filter treated with BioBrene

Step 4 — Load Sample onto the Glass Fiber Filter

To remove, disassemble and clean the reaction cartridge(s):

1. Unscrew and remove the reagent inlet cap connected to the protein sequencer.
2. Remove the reaction cartridge from the holder.
3. Unscrew and remove the cartridge block holder cap (Figure 3-1 on page 3-11).
4. Slowly invert the cartridge block holder until the upper and lower glass cartridge blocks slide out.
5. Place the upper cartridge block on a clean, dry surface with the precycled filter facing up.
6. Discard the used PROCISE cartridge seal.
7. Clean the *lower glass cartridge block only* by rinsing the inner surface with methanol.
8. Place the lower glass cartridge block in the cartridge block drying assembly on the protein sequencer, and dry it with a stream of argon.

Loading Sample

Load your sample onto the center of the treated glass-fiber filter, so it distributes evenly across the filter.

Note: The maximum liquid capacity of a dry 6 mm filter is approximately 7.5 µL, a 9 mm filter is approximately 15 µL. With very dilute samples, you may need to load more than the maximum total volume. Additional fluid can be loaded by drying the filter between loadings.

To reassemble and leak test the reaction cartridge(s):

1. Referring to Figure 3-2 on page 3-11, place the Teflon seal back into the cartridge block holder if it came out during disassembly.
2. Insert the lower glass cartridge block in the cartridge block holder.
3. Using forceps, place a new cartridge seal on top of the lower glass cartridge block in the holder.
4. Hold the lower glass cartridge block and PROCISE cartridge seal in place inside the holder by placing your fingers in the cartridge block holder windows. Invert the holder as shown in Figure 3-5 on page 3-13.
5. Slide the upper glass cartridge block with the sample up into the cartridge block holder until it is flush against the lower cartridge block.
6. Invert the holder once again so it is upright, and screw on the cartridge block holder cap until snug.

7. Place the reaction cartridge into the cartridge assembly on the protein sequencer.
8. Reassemble the cartridge assembly:
   a. Insert the completed block holder in the cartridge assembly.
   b. Place the cap on top of the assembly, align the guide pins and slots, then press down and turn the cap until it stops against the body of the assembly.

   Note: The seal between the cartridge blocks and the Kel-F ferrules is made by spring force. Overtightening the cartridge assembly does not increase the sealing force.

Step 5 — Perform a Cartridge Leak Test

Repeat the cartridge leak test by following the instructions on page 3-14.

You are now ready to sequence your sample.

Determining the Number of Sequencing Cycles

Overview

Each repetition of the Default sequencing cycle yields one residue. Determine the number of residues needed and add the number of residues to the number of preparation cycles in the method you are using.

Note: The standard sequencing methods (Table 3-1 on page 3-6) each have three preparation cycles — cycles 1, 2 and 3.

Example

For a sequencing yield of seven residues from Cartridge A only, using the Pulsed-Liquid sequencing method. Configure Cartridge A in the Start Run dialog box (Figure 3-7) as follows:

To define a sequencing yield:

1. Select 1st for the Run Order.
2. Enter a unique sample name in the File Name box.
3. Specify **10** in the Cycles box.
4. Select **Pulsed-Liquid** for the sequencing method from the Method pop-up menu.
5. Enter the amount of sample and standard in pmol in the appropriate boxes.

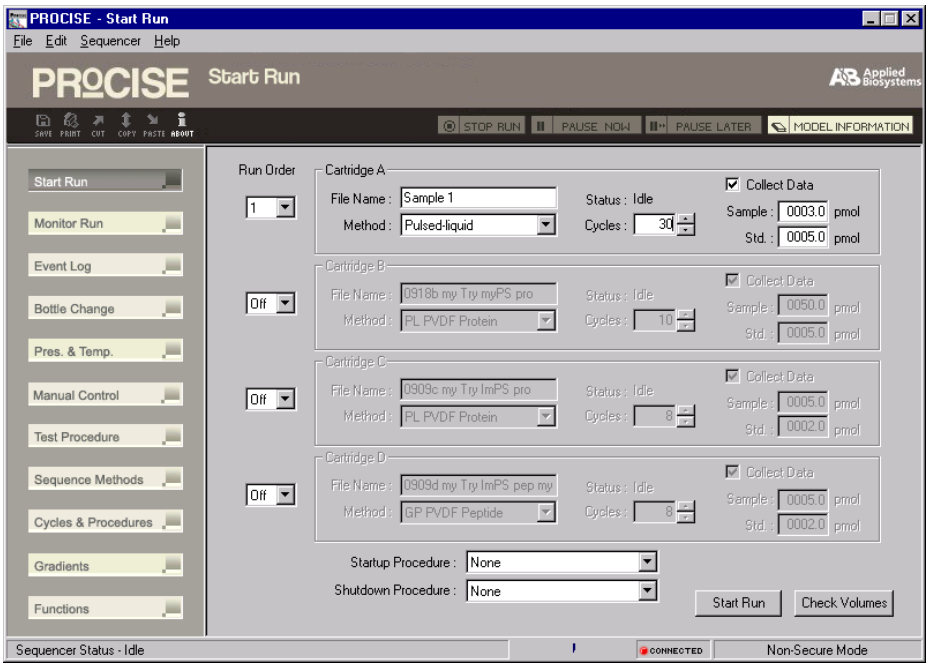


Figure 3-7 Configuring the Start Run dialog box

Starting a Run

Before the Run

WARNING **CHEMICAL WASTE HAZARD.** Do not remove the waste bottle when the protein sequencer is running because fluid and vapors are actively delivered to the waste bottle during a run. Waste produced by this system can be hazardous and can cause injury, illness, or death. Only operate a vented instrument if it is connected in accordance with all the requirements. Handle all liquid, solid and gaseous waste as potentially hazardous. Protein sequencer waste must be disposed of properly and carefully in accordance with all state, local, and federal requirements. Refer to the waste profile in the PROCISE/PROCISE *cLC Site Preparation and Safety Guide (P/N 4314377)* for classification of waste before disposal. When handling the waste for disposal, wear gloves and use eye protection. Avoid inhalation and skin contact.

Before proceeding with the following instructions, you should have:

- Loaded the sample(s) onto the protein sequencer
- Leak tested all loaded reaction cartridges

Starting a Sequencing Run

To start a sequencing run:

1. Perform the actions listed in the Pre-run Checklist.
2. Purge the ABI 140 pump. See “Purging the 140 Pump with Fresh Solvent” on page 2-13.
3. Set up the protein sequencer for a run.

Pre-run Actions Checklist

- Check the quantities of sequencing chemistry and HPLC solvents. Replace chemistry and solvents as necessary, to ensure sufficient quantities are present for the entire run. The bottle change procedure is listed in Chapter 2, “Preparing the System.” See “Checking Volumes” below to estimate the amount of reagents and solvents needed for a run.

IMPORTANT! Changing HPLC solvents during sequencing can cause retention times to shift, and make peak identification difficult.

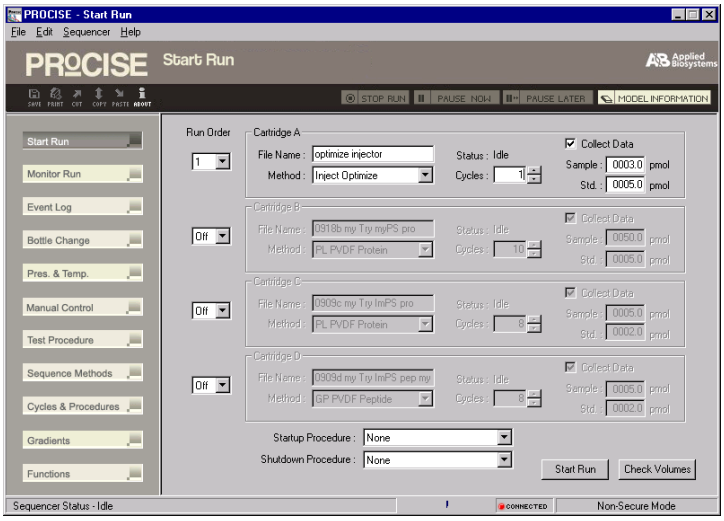
- Check the protein sequencer and 140 pump waste bottle levels. Empty the bottles if the waste level is close to 2 in. from the top of the bottle. See “Emptying the Waste Bottle on the Instrument” on page 2-28.
- Check the argon supply. Enough argon must be present for the entire run. Change the argon tank if necessary. See “Removing the Argon Cylinder” on page 2-29.

Checking Volumes

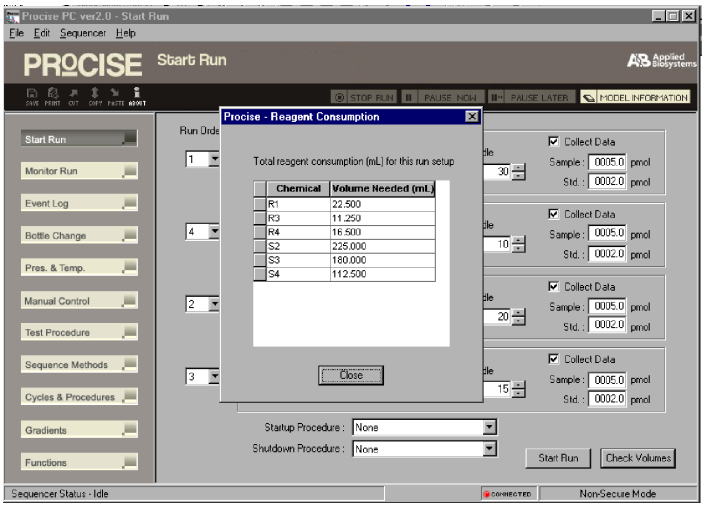
Use the Check Volumes feature to estimate the volume of reagents and solvents needed for the run.

To check volumes:

1. Select **Start Run** to open the Start Run window.



2. Enter the cartridges, methods, and cycles that will be run.
3. Click **Check Volumes** to open the Procise- Reagent Consumption dialog box that lists the reagents, solvents, and approximate volumes needed to complete the run.



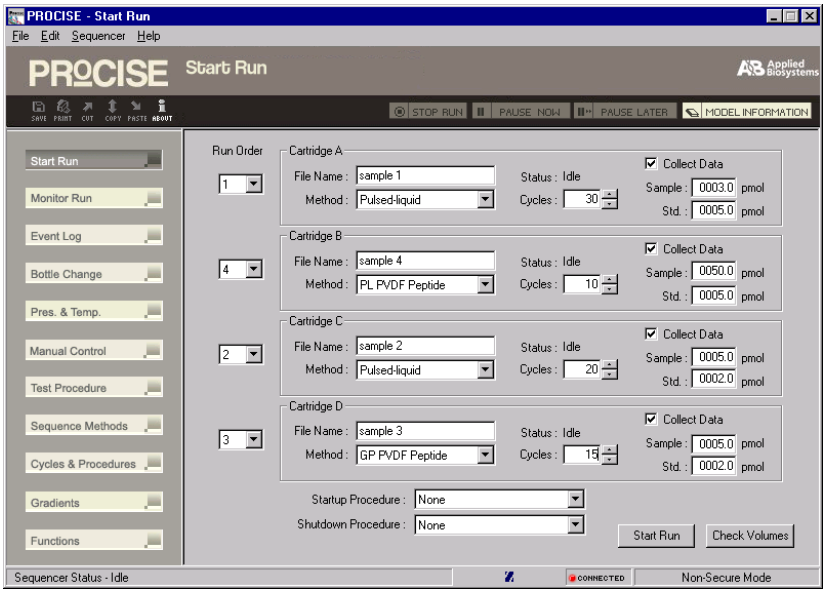
Set Up the Protein Sequencer for a Run

Before starting a run, always perform a cartridge leak test on each cartridge you have loaded.

The cartridge leak test is included as part of the sample loading procedures on the preceding pages of this section. If you have loaded your sample(s), but have not run this test, turn to page 3-23 now, and run the cartridge leak test.

To set up the protein sequencer for a run:

1. If the system has been idle for one or more days, refer to Chapter 7, "Maintenance." for information and instructions on the procedures you should run before sequencing a sample.
2. Select the Start Run dialog box from the dialog box pop-up menu.



3. Select the cartridge run order. Cartridges can be run in any order. Selecting the run order for a cartridge activates the File name, Cycles and Methods fields.
4. Enter a unique file name for each sample.
5. Enter the number of cycles to be run by highlighting each cycle field and typing the number, or by using the scroll up/down button.

   Note: For filter precycling, enter 5 or more cycles as appropriate. When sequencing samples using the standard methods, the first 3 cycles prepare the protein sequencer and sample for sequencing. Therefore, if 20 residues are required, enter 23 in the Cycles box.
6. Open the Method pop-up menu and select the appropriate method for each cartridge.
7. Select the Collect Data boxes if they are not already selected. An X will appear in the box when selected.
8. Enter the sample and standard amounts to be run for each cartridge.
9. Click **Start Run**, or press **Return**.

Next Step

After you have performed these steps:

- Sequencing parameters are downloaded to the protein sequencer and the 140 pump.
- The Monitor Run window is displayed.

How Data Is Collected During a Run

The PROCISE Systems contain a virtual analog-to-digital (A/D) converter. The maximum storage capacity of the converter is 90 minutes of data.

The SequencePro™ Data Analysis Application software controls data collection from the converter. When a sequencing run begins, a virtual A/D file is created in the PROCISE Folder located in the Winnt Folder.

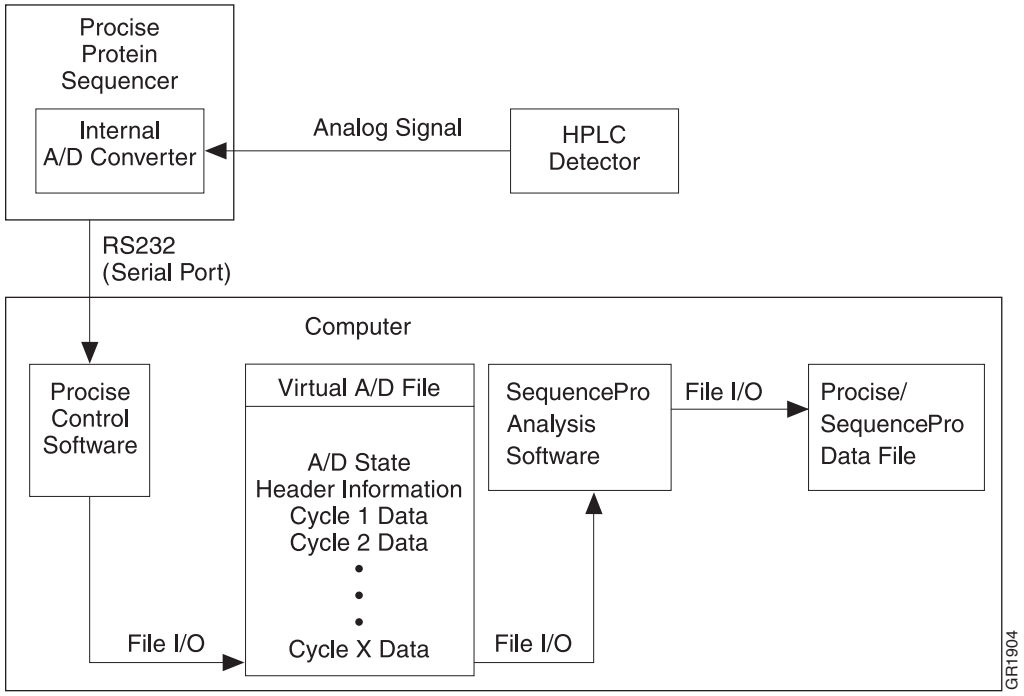


Figure 3-8 Virtual A/D data collection

Data Collection

Throughout the run, data is collected and appended to the virtual A/D file. When the run is complete:

- The control software turns on the Run Finished flag in the virtual A/D file. This flag indicates that the file contains a complete set of sequencing data.
- The virtual A/D file contains:

  Complete header information (protein sequencer name; sample name; run data and time; sample and standard amount).

  The raw data for each cycle in the sequencing run for the specified cartridge and sample.

You can start the SequencePro software at any time. The software continuously monitors the protein sequencer folder for new virtual A/D files.

Chromatogram Display Window

A chromatogram display window is opened for each virtual A/D file, and the data is stored in a PROCISE/SequencePro data file. The data file and the chromatogram are updated every 15 sec until the run is finished and all the data is collected.

When the run is finished, the virtual A/D file is deleted, and the SequencePro data file is stored in a location designated by the SequencePro preferences.

Preventing Data Loss

If the protein sequencer determines that the control software has stopped collecting data from converter, the protein sequencer pauses automatically at the end of the current cycle, and waits until data collection resumes. This mechanism helps prevent data loss.

Optimizing Sequencer Processes 4

This chapter describes the optimization processes that must be performed to ensure consistent chromatography.

The following topics are contained in this chapter:

Injector, and Sensor Functions/Sensor Failure Event Log Messages

Injector

Reconstituting the Sample

During flask cycles, a sample is reconstituted in 10% acetonitrile (S4C) or 20% acetonitrile (S4B, C9). The acetonitrile ensures that hydrophobic amino acids go into solution. For consistent chromatography, however, the percentage of acetonitrile must be reduced before sample injection.

Reducing Acetonitrile Content

To reduce acetonitrile content, one or two Concentrate Sample steps are included in all flask cycles. The duration of these steps determines the percentage of sample injected. Therefore, the duration of the Concentrate Sample step(s) must be optimized so that the correct amount of sample is injected.

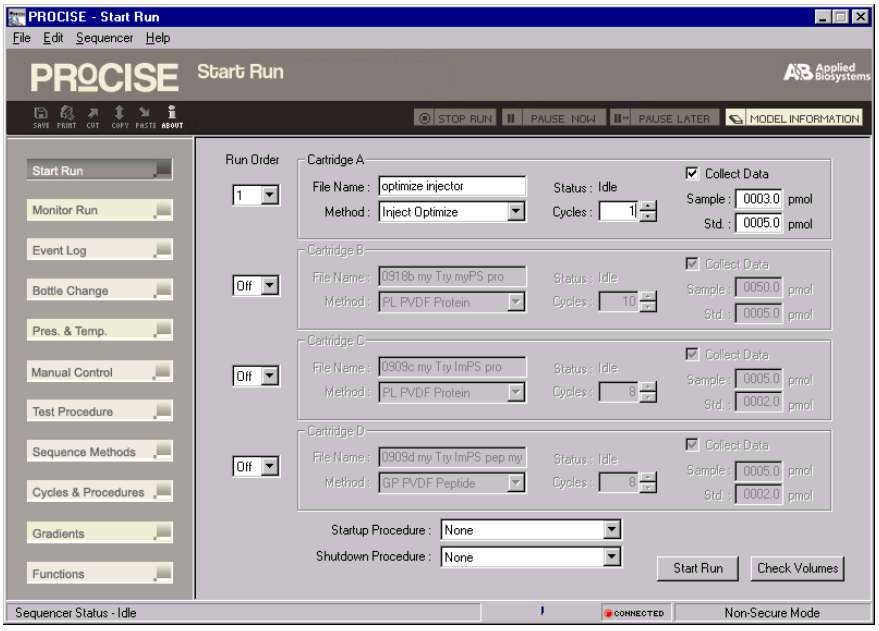
Optimize the injector percentage if Sample Loop Full error messages are recorded in the event log.

Setting Up the Procise cLC Protein Sequencer

Two procedures are provided here, one to set up the protein sequencer for optimization and the second to perform optimization.

To set up the Procise cLC protein sequencer for optimization:

1. Scroll to the Functions dialog box.
2. Ensure that the global time for the Concentrate Sample step (fn 238) is 100 sec.
3. Place a mark 1 in. from the hexagonal tip of the 5/16-in. bushing on the yellow tubing connected to valve block port 42.
4. Scroll to the Start Run dialog box.



5. Set up a run with the following conditions:

Parameter	Setting
Cartridge A	1st
Filename	Your choice
Number of cycles	10
Method	Injector Optimization

To run the optimization procedure:

1. Click **Start Run**.

   The Init Sensor procedure will start running.

2. If the flask temperature is 64 ˚C when the run pauses at the Begin step of the flask cycle, click Next Step to start the Injector Optimization method.

3. Click Pause Later, and configure the run to pause on Cartridge A at the end of the first cycle.

4. At the end of the first cycle, look for the injection slug in the plumbing line.

   Ideally, the *end* of the slug should be between the mark you made on the tubing and the valve block. It should not be in the pickup line connected to port 41.

5. If the end of the injection slug is not in the correct location, modify the Concentrate Sample time global value in the Functions dialog box. Increase or decrease the value as appropriate in **5-sec increments only**.

   IMPORTANT! Do not increase the Concentrate Sample step time by more than 5 sec at a time. Otherwise, an air injection might occur. An air injection will damage the column.

6. Click **Resume**.

7. Once you have determined the correct value for the Concentrate Sample step, run at least one more cycle to confirm the optimization.

Sensor Functions

About Fluid Sensor Functions

Sensor functions control protein sequencer valves for reagent and solvent delivery. Each fluid sensor function is controlled by one of the 11 optical fluid sensors in the protein sequencer.

Four Function Types

There are four types of sensor functions:

- Cartridge load functions
- Deliver to cartridge functions
- Flask load functions
- Injector load functions

How Optical Fluid Sensors Work

Each sensor consists of an infrared emitting diode and photosensor receiver. When fluid is present, the sensors detect a change in refractive index due to increased light transmission through the Teflon™ tube.

List of Optical Fluid Sensors

There are 11 optical fluid sensors in the protein sequencer:

- Cartridge Load 1 (Small) sensor
- Cartridge Load 2 (Large) sensor
- Cartridge A Outlet sensor
- Cartridge B Outlet sensor
- Cartridge C Outlet sensor
- Cartridge D Outlet sensor
- Flask Load 1 (Small) sensor
- Flask Load 2 (Large) sensor
- Transfer to Flask sensor
- Sample Loop Load sensor
- Sample Loop Full sensor

How Sensor Functions Work

When a sensor function is activated:

- The sensor begins "looking" for fluid
- The function timer begins counting down to zero

If...	Then...
fluid is detected	1. The system turns off the reagent or solvent delivery valve. 2. The injector switches from the load position to the inject position.
the timer reaches "0"	the next step begins. **Note:** The function must remain active long enough for fluid to reach the sensor. How long the function remains active is specified when the function is created.
fluid is not detected within the specified period of time	• A dialog box describing the failure(s) is displayed for each sensor (except the Transfer to Flask and Sample Loop Full sensors). • The protein sequencer pauses at the end of the active cycle (unless the operator intervenes). • An error message is sent to the event log for all sensor failures, including the Transfer to Flask and Sample Loop Full sensors.

Reading Sensor Failure Event Log Messages

Typical Sensor Failure Message

Figure 4-1 is a typical Event Log message for a sensor failure.

```
01/01/1995          4:30:00 PM
          During step 2 of cycle 1, fluid was not detected by the Cartridge Load 2 (large) Sensor
          The sequencer will pause at end of this cycle.
(Dry = 500, Threshold = 750, Average wet = xx)
          dry     wet     dry     wet     dry     wet
          (xx,    xx,     xx,     xx,     xx,     xx,
           xx,    xx,     xx,     xx,     xx,     xx,
           xx,    xx,     xx,     xx,     xx,     xx)
```

Figure 4-1 Typical event log message for a sensor failure

Sensor Failure Message Information

The following information is displayed in this event log message:

- The date and time the failure occurred
- The step and cycle number during which the failure occurred
- The sensor that reported the failure
- The status of the protein sequencer

Note: A full explanation of the line "(Dry = 500, Threshold = 750, Average wet = xx)" in Figure 4-1 is given in Table 4-1.

Dry and Wet Values Overview

The six columns of dry and wet values represent the number of dry and wet readings taken by the sensor.

IMPORTANT! A certain number of wet readings are required to discriminate the arrival of the reagent or solvent from a stray droplet of fluid in the line. If fluid never reaches the sensor, only the first dry field will have a nonzero value.

Definitions of Dry and Wet Values

Table 4-1 Definitions of Dry, Threshold, and Average Wet values

Value	Definition
Dry = 500	The empty tube transmission (dry) reading from the sensor generated during the Init Sensor procedure.
Threshold = 750	The minimum transmission value necessary for a sensor reading to be considered wet (dry reading multiplied by 1.5).
Average wet = xx	Actual transmission reading with fluid in tube. **If no fluid is detected, the average wet = 0.**

Why Sensor Failure Event Log Messages Are Generated

Causes of Sensor Failure Event Log Messages

Sensor failure event log messages are generated under any of three conditions:

- A bottle runs dry during a run.
- A delivery path blockage restricts the flow of a reagent or solvent.
- Air bubbles are present in the solvent or reagent stream.

Bottle Runs Dry or Blockage Event Log Error Message

If a bottle runs dry or a blockage occurs, an Event Log error message such as the one shown in Figure 4-2 is generated.

```
dry     wet     dry     wet     dry     wet
(5000,  0,      0,      0,      0,      0,...)
```

Figure 4-2 Event Log message indicating an empty bottle or restricted delivery of a reagent/solvent

Air Bubbles Event Log Error Message

When air bubbles are detected by a sensor, an Event Log message with values such as those shown in Figure 4-3 is generated.

```
dry     wet     dry     wet     dry     wet
(1500,  58,     1,      47,     2,      53,...)
```

Figure 4-3 Event Log message reporting bubbles in the solvent/reagent stream

IMPORTANT! Bubbles occur when the solvent or reagent degasses as it flows through the valve blocks. This condition can usually be corrected by reducing the appropriate bottle pressure.

Cartridge Sensor, Delivery to Cartridge Sensor, and Flask Load Sensor Functions

Cartridge Load Sensor Functions

Two load loops are available for metering reagents to the cartridge:

- Small loop
- Large loop

Load Loop Details

The two load loops are described in Table 4-2:

Table 4-2 Load loop descriptions

Loop	Description
Small	• Loads a nominal 5 µL of any cartridge reagent. • The volume of reagent delivered wets, but does not saturate, a 6-mm-glass fiber filter in the reaction cartridge. • The volume of reagent delivered is appropriate for blotted samples. • May be preferred when you are sequencing samples on small pieces of PVDF. **Note:** A small loop load method for TFA, such as pulsed-liquid cLC, can help prevent sample washout from occurring.
Large	• Loads a nominal 10 µL of any cartridge reagent. • Delivers a volume of reagent that saturates a 6-mm-glass fiber filter in the reaction cartridge.

Load Loop Guidelines

The standard cycles included with the PROCISE Protein Sequencer use large and small load loops for loading cartridge reagents.

If...	Then...
this is the first loading	flush the loop for at least 15 sec before the first loading.
you are between loadings	flush the loop for at least 10 sec.
you are loading multiple reagents	wash and flush the loop between loadings.
the delivery pressure for a reagent is changed	change the load times as well.
the protein sequencer has not been run since the last cold start	run the Init Sensor procedure from the Test dialog box. Allow the procedure to run to completion to ensure proper sensor operation.

Calculating Function Duration

Follow the steps below to determine the duration required for a Cartridge Load sensor function.

Note: All manual control functions and valves must be deactivated before procedures or runs can be started.

To determine the duration:

1. From the Pressures and Temperatures dialog box, set the delivery pressure for the appropriate bottle position.
2. If the reagent or solvent is not loaded on the protein sequencer, load it using the Bottle Change procedure in Chapter 2, "Preparing the System."
3. Select the function:
   a. From the Manual Control dialog box, select the appropriate function from the cartridge function list (Function 139, Flush Large Loop, or Function 140, Flush Small Loop).
   b. Activate the function for 20 sec.
4. Activate the Load function for the bottle and loop.

   For example, select Function 183 to load the large loop with reagent or solvent from the X2 (C5) bottle position.
5. Monitor the Reaction Flow sensor.
   a. Watch for the appearance of a check mark next to the Reaction Flow Sensor field at the top of the screen.
   b. Note the elapsed time, and add 5 to 10 sec for the load time.
6. Enter the load time in the cycle for this function.
7. From the Functions dialog box, enter the load time in the Global Time field for that function.
8. Save the change by opening the File menu and selecting **Save Function**.

Delivery to Cartridge Sensor Functions

Fluid sensors simplify the optimization of solvent delivery to cartridges for washing and extraction. The sensors are located at the cartridge outlets.

The sensors eliminate the need for timing the delivery of solvent to the midpoint of the cartridge.

Washes and Extractions

All washes and extractions in standard cycles are controlled by fluid sensors (except for the wash after cleavage).

A wash is a two-part procedure:

- A delivery to the cartridge outlet sensor
- Short pulses of solvent occurring, alternating with wait steps

An extraction is also a two-part procedure:

- Deliveries to the cartridge outlet sensors
- A brief incubation period followed by transfer to the flask

IMPORTANT! Whenever the delivery pressure for a reagent is changed, load times must also be changed.

Guidelines for Using Deliver to Cartridge Sensor Functions

If...	Then...
this is the first delivery	flush the cartridge for at least 40 sec before the first delivery.
you are between deliveries	flush the cartridge for at least 40 sec.
the protein sequencer has not been run since the last cold start	run the Init Sensor procedure from the Test dialog box. Allow the procedure to run to completion to ensure proper sensor operation.

Determining the Function Duration

To determine the duration required:

1. From the Pressures and Temperatures dialog box, set the delivery pressure for the appropriate bottle position.
2. If the reagent or solvent is not loaded on the protein sequencer, load the bottle using the Bottle Change procedure in Chapter 2, “Preparing the System.”
3. From the Manual Control dialog box, select Function 131, Dry Cart (top), from the cartridge function list. Activate the function for 40 sec.
4. Activate the Deliver to Cartridge (sensor) function for the bottle or solvent.

   For example, select Function 75, Load X1 (C6), Cart (sm loop), to deliver reagent or solvent from the X1 (C6) bottle position to the cartridge outlet sensor.
5. Note the time that has elapsed.
   a. Watch for the appearance of a check mark next to the Reaction Flow Sensor field at the top of the screen.
   b. Note the elapsed time, and add 5 to 10 sec for the load time.

6. Enter the load time in the cycle for this function.
7. From the Functions dialog box, enter the load time in the Global Time field for that function.
8. Save the change by opening the File menu and selecting Save Function.

Note: All manual control functions and valves must be deactivated before procedures or runs can be started.

Flask Load Sensor Functions

Either of two load loops are available for the flask. Unlike cartridge load loops, the volume of any particular reagent or solvent loaded depends on the position of that chemical on the valve block. Nominal volumes are listed in Table 4-3.

Table 4-3 Nominal volumes of reagents/solvents for small and large loops

Bottle Position	Small Loop (µL)	Large Loop (µL)
S4 (C9)	25	60
X3	20	55
X2 (C5)	15	50
R4	10	45
R5 (C1)	5	40

Using Flask Load Sensor Functions

If...	Then...
this is the first loading	flush the loop for at least 10 sec before the first loading.
you are between loadings	flush the loop for at least 10 sec.
you are loading multiple reagents	wash and flush the loop.
the delivery pressure for a reagent is changed	change the load times as well.
the protein sequencer has not been run since the last cold start	run the Init Sensor procedure from the Test dialog box. Allow the procedure to run to completion to ensure proper sensor operation.

PROCISE and PROCISE cLC Chromatography

This section describes the procedures used for the PROCISE® liquid chromatography (HPLC) system. Instructions for preparing solvents and optimizing the separation of PTH amino acids are in this section. Routine operations of the PROCISE HPLC system are controlled by PROCISE control software. For complete descriptions of the menus used to control the pumps, refer to the *Model 140C Micro gradient Delivery System User's Manual.*

HPLC Solvents

About the HPLC Solutions

WARNING CHEMICAL HAZARD. A3 (3.5% tetrahydrofuran in water) is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation, central nervous system depression, and liver and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING CHEMICAL HAZARD. B2 (12% isopropanol and acetonitrile) is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING CHEMICAL HAZARD. Acetonitrile (ACN) is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DANGER CHEMICAL HAZARD. Premix Buffer Concentrate causes burns to the eyes, skin, and respiratory tract. It is a combustible liquid and vapor. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Solvent A3

The composition of Solvent A3 is 3.5% tetrahydrofuran/water. Together with the Premix buffer concentrate, Solvent A3 provides optimal separation of the PTH-amino acids.

Solvent B2

Solvent B2 contains 12% isopropanol in acetonitrile to resolve PTH-Trp from the chemical artifact, diphenylurea (DPU).

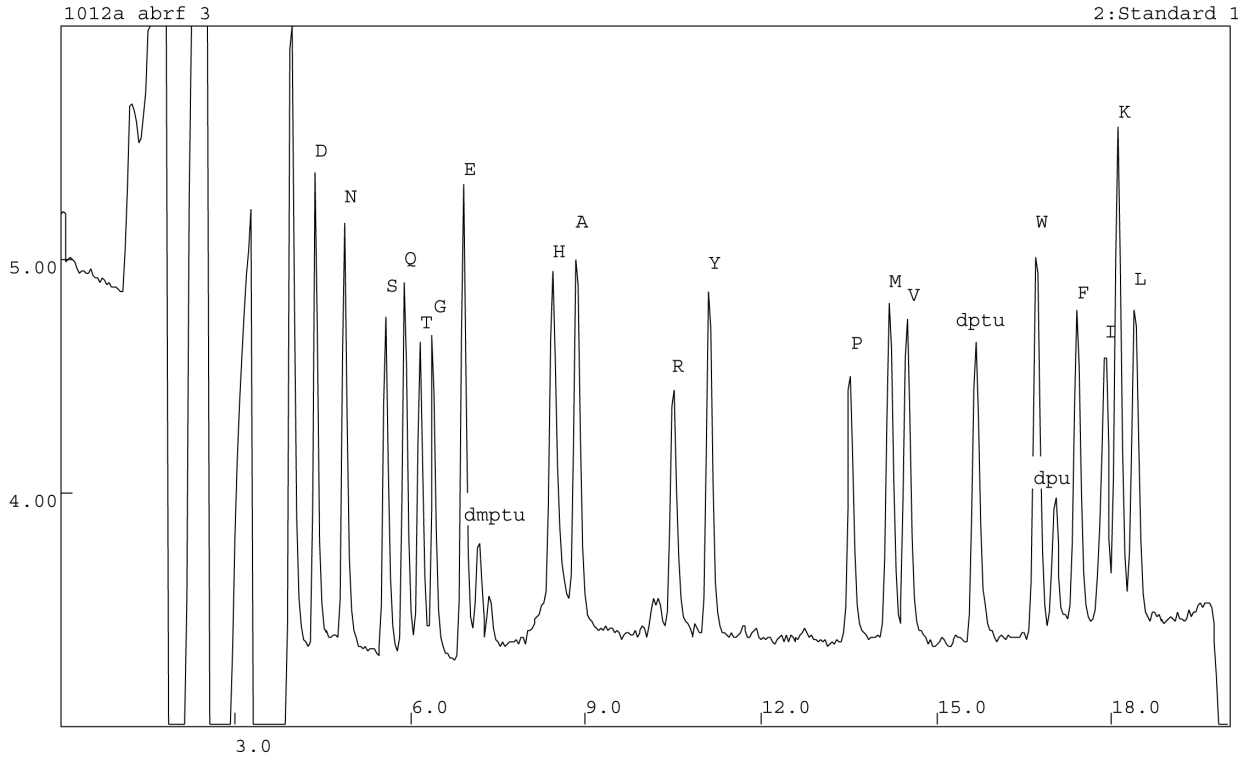


Figure 4-4 Five pmoles of PTH-amino acid standard was analyzed using solvent B2 and solvent A3 containing Premix Buffer Concentrate. Notice that the DPU is favorably positioned between Trp and Phe.

Premix Buffer Concentrate

The premix buffer employs an ion-pairing additive to improve both peak shape and retention time reproducibility for the PTH-derivatives of histidine, arginine and the pyridylethyl derivative of cysteine. PTH-derivatives with positively-charged side-chain groups interact with underivatized silanol groups on the silica particles in a column causing peak broadening and retention time shifting. By adding an ion-pairing modifier to the mobile phase, the interaction of the basic derivatives with free silanol is significantly reduced through preferential interaction with a strongly acidic ion-pairing additive.

Adding Premix Buffer

Influence the separation by adding Premix buffer.

If...	Then...
the desired elution order is: PTH-His before PTH-Ala, PTH-Arg before PTH-Tyr, and PTH-PE-Cys before PTH-Pro	add ~25 mL of Premix buffer concentrate (P/N 401446) to 1 liter of solvent A3, 3.5% THF(P/N 401464). Cap and mix well.

If...	Then...
the PE-Cys is not a derivative of interest	it is also possible to position His after Ala and Arg after Tyr by using less Premix buffer. Approximately 10 mL of Premix will usually give good separation with His and Arg in these later elution positions (see Figure 4-5).

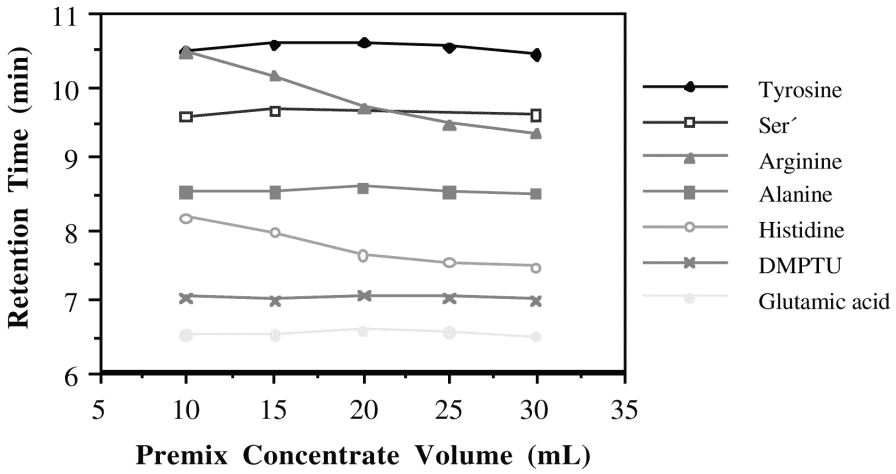


Figure 4-5 Effect of Premix Buffer concentration on retention time

Flattening the Baseline

In order to achieve a high sensitivity sequence, it is critical to minimize any baseline rise. One factor that causes the baseline rise in PTH chromatograms, is the slightly higher absorbance properties of solvent B2. Eliminating this problem can increase accuracy in computer integration at high sensitivity.

Acetone has a very high UV absorbance at 269 nm, a wavelength that is optimal for PTH amino acid analysis. When small amounts of acetone are added to the solvent A3 and a linear gradient is implemented, the absorbance of solvent A3 and solvent B2 will match, therefore most of the baseline rise will be eliminated. See “About the HPLC Solutions” on page 4-11 for warnings about B2, A3, and acetone.

To flatten the baseline by adding acetone to Solvent A3:

1. Make 1% acetone/H_2O solution:

   Mix 1 mL of HPLC grade acetone and 99 mL of milli-Q water in a 100 mL clean bottle.

2. Add 1 mL of 1% acetone/water to 1 liter of solvent A3 buffer or 450 µL of 1% acetone/water to 450 mL of solvent A3 buffer. Mix well.

Reducing Negative Baseline Slope at the Start of the Chromatogram

Some HPLC and/or PTH-columns may exhibit a negative slope in the baseline from DTT to Glu before flattening out in the latter part of the chromatogram. The addition of a small amount of phosphate ion to Solvent A usually reduces or eliminates this problem.

To reduce a negative baseline by adding phosphate ion to Solvent A:

1. Prepare a 1.0 M stock solution of NaH_2PO_4 or KH_2PO_4 (monobasic sodium or potassium phosphate, sodium or potassium dihydrogen phosphate).
2. Add 45 to 100 µL of the phosphate solution to Solvent A to provide a final concentration of 0.1 mM phosphate.

Note: Adding a phosphate solution flattens the baseline over several cycles and prevent reappearance of the slope.

PROCISE PTH-Amino Acid Separation

PTH-AA Separation at Installation

The steps for optimizing the separation of specific PTH-amino acids and chemistry artifacts are described in this section.

During installation, the PTH-amino acid separation is optimized for the PTH column supplied with your instrument. The gradient used is stored in the PROCISE control software. These conditions serve as the starting point. To maintain optimum separation, the gradient may require fine-tuning as the column ages.

Positioning the Positively Charged PTH-AAs

The positively charged PTH-AAs, are:

- Histidine
- Arginine
- Pyridylethyl cysteine

Increasing the ionic strength of the mobile phase reduces the retention of the basic derivatives on the column. Suggested elution positions for the basic derivatives are His between DMPTU and Ala, Arg between Ser´ and Tyr, and PECys before Pro.

For the majority of columns, these elution positions can be obtained by using ~20 mL of the Premix buffer concentrate per liter of solvent A3, and making minor gradient modification. However, increasing the buffer concentration can be a useful method to make Arg elute earlier than Ser´ and to make PECys elute earlier than Pro.

If...	Then...
PECys is not a derivative of interest	it is possible to position His after Ala and Arg after Tyr by using less Premix buffer. Approximately 10 mL of Premix will usually give a good separation with His and Arg in these later elution positions.

Histidine

If...	Then...
His coelutes with Ala	increase the buffer concentration.
His needs to move before Ala	add an additional 5 mL of Premix buffer concentrate per liter of Solvent A3.

Arginine

If...	Then...
Arg coelutes with Tyr	increase the buffer concentration.
Arg needs to move before Tyr	1. add an additional 5 mL of Premix buffer concentrate per liter of solvent A3. 2. Increase the %B at 0.4 minutes to 14–16%.
the Ser´ coelutes with the Arg peak	improve the separation by shifting the Ser´ peak before Arg by: 1. Decreasing the column temperature 2–5 °C. Adjust the temperature carefully because the separation of Met from Val is reduced by decreasing the column temperature. 2. Decreasing the flow rate. A decrease in flow rate from 325 mL/min–300 mL/min moves Arg slightly relative to Ser´ without significantly impacting any other separations. The %B at 18 minutes should be decreased by 1% to maintain optimum separation of Ile/Lys.

Pyridylethyl Cysteine

If...	Then...
PECys coelutes with Pro	increase the buffer concentration. Add approximately 5 mL of Premix buffer concentrate per liter of Solvent A3 to move PECys before Pro.

The Acidic Amino Acids

The acidic amino acids are:

- Aspartic acid
- Glutamic acid

Aspartic Acid

If...	Then...
Asp needs to be separated from the DTT peak	decrease the initial %B. If the initial %B is reduced below 8%, DMPTU will probably move under Glu. (The DTT peak elutes immediately after the negative dip of the injection artifact.) Add a gradient step at 0.4 minutes.

If...	Then...
decreasing the initial %B causes Glu to coelute with DMPTU	add a gradient step at 0.4 minutes and set the %B to 14–16%. The initial %B can then be lowered below 5% without losing the Glu/DMPTU separation. **Note:** Decreasing the initial %B may cause Asp and phenylthiourea (PTU, a reaction product of PITC and NH_3) to coelute.
the gradient adjustments fail to separate Asp from the DTT peak	add 100 µL of neat TFA per liter of Solvent A.
Asp needs to be separated from phenylthiourea (PTU, reaction product of PITC and NH 3)	1. Increase initial %B. Increasing the initial %B to 8–10% will usually separate the Asp peak before PTU, moving it towards the DTT peak. (The DTT peak elutes immediately after the negative dip of the injection artifact.) 2. Decrease the flow rate. A decrease in flow rate from 325 mL/min–300 mL/min will move Asp away from PTU without significantly impacting any other separation. The %B at 18 minutes should be decreased by 1% to maintain optimum separation of Ile/Lys.

Glutamic Acid

If...	Then...
Glu needs to be separated from DMPTU	increase the initial %B. If the Asp/DTT peak separation will not be compromised, increase the initial %B by 2–3%. add a gradient step at 0.4 minutes. If increasing the initial %B will cause the Asp to elute too early, add a gradient step at 0.4 minutes and set the %B to 14–16%.

Improving the Separation of Other Amino Acids

To improve the...	Then...
Met/Val separation	increase the column oven temperature. Increase the temperature in 2 °C increments. Do not raise the temperature above 59 °C.
Ile/Lys separation	decrease the %B at 18 minutes If the peaks are more than 50% separated, decrease the %B by 1%. If the peaks are less than 50% separated, decrease the %B by 2%.
Lys/Leu separation	increase the %B at 18 minutes. If the peaks are more than 50% separated, increase the %B by 1%. If the peaks are less than 50% separated, increase the %B by 2%.

Optimization Table

In Table 4-4, the arrow above an amino acid indicates the direction in which the peak moves after changing the variable listed in the Variable column. Left is toward the injection point.

Table 4-4 Optimization guidelines with Procise System

Variable	Major Effect	Minor Effect
Increase initial %B	← ← DTT D PTU	← E DMPTU ← H ← S´ R
Decrease initial %B	→ → DTT D PTU	← S → Q → E DMPTU → H
Increase %B at 0.4 minutes	← E DMPTU ← H ← S´ R	← S ← Q T
Decrease %B at 0.4 minutes	→ E DMPTU → H	
Increase final %B at 18 minutes	← I K L	
Decrease final %B at 18 minutes	→ I K L	
Decrease pH	→ DTT D	→ G E
Increase column temperature (2 °C)	← → M V	← H ← R ← PECys
Decrease column temperature (2 °C)	→ S´ R → ← M V	T → G
Increase molarity	← H A ← R S´	← PECys P
Fast Gradients Only: Decrease flow rate	→ D PTU S´ → R	

PROCISE cLC PTH-Amino Acid Separation

Modifying the Standard Gradient Program

During installation, PTH-amino acid separation is optimized for the column supplied with your system using the standard gradient program, Normal 1 cLC. To maintain optimum separation, you may need to modify this program as the column ages.

Positioning Positively Charged PTH–Amino Acids

Increasing the ionic strength of the mobile phase reduces the retention time of the basic derivatives on the column. Suggested elution positions for the basic derivatives are:

- Arginine between serine´ and tyrosine
- Pyridylethyl cysteine before proline

If...	Then...
you want arginine between serine and tyrosine Pyridylethyl cysteine before proline	Do this in two ways: • Add approximately 9–11 mL of Premix buffer to 450 mL of solvent A3 • Making minor gradient program modifications
you want arginine eluting earlier than serine, and pyridylethyl cysteine eluting earlier than proline	increase the buffer concentration.
pyridylethyl cysteine is not a derivative of interest, you can position histidine after alanine, and arginine after tyrosine	by using less Premix buffer.

Histidine

If...	Then...
histidine coelutes with alanine	increase the buffer concentration.
moving histidine before alanine	add an additional 1 mL of Premix buffer per 450 mL of solvent A3.

Arginine

If...	Then...
arginine coelutes with tyrosine	increase the buffer concentration.
moving arginine before tyrosine	add an additional 1 mL of Premix buffer to 450 mL of solvent A3.

Pyridylethyl Cysteine

If...	Then...
pyridylethyl cysteine coelutes with proline	increase the buffer concentration.
moving pyridylethyl cysteine before proline	add approximately 1 mL of Premix buffer to 450 mL of solvent A3.

Improving the Separation of the Aspartic Acid

If...	Then...
separating aspartic acid from the DTT peak	add 20 µL of neat trifluoroacetic acid to 450 mL of solvent A3.

Improving the Separation of Other Amino Acids

The following amino acids can be separated:

- Methionine/Valine
- Isoleucine/Lysine
- Lysine/Leucine

Methionine/Valine

If...	Then...
separating methionine from valine	increase the column temperature in 2 °C increments. Do not raise the temperature above 59 °C.

Isoleucine/Lysine

If...	Then...
the peaks are more than 50% separated	decrease the %B by 2% at 22 min.
the peaks are less than 50% separated	decrease the %B by 4% at 22 min.

Lysine/Leucine

If...	Then...
the peaks are more than 50% separated	increase the %B by 2% at 22 min.
the peaks are less than 50% separated	increase the %B by 4% at 22 min.

Summary of PTH-Amino Acid Separation Optimization

In Table 4-5, the arrow above an amino acid indicates the direction in which the peak moves after changing the variable listed in the Variable column. Left is toward the injection point.

Table 4-5 Effect of variables on baseline peaks with the Procise cLC System

Variable	Major Effect	Minor Effect
Increase final %B at 22 min.	← I K L	
Decrease final %B at 22 min.	→ I K L	
Increase column temp (2 °C)	← → M V	← ← H R PECys
Decrease column temp (2 °C)	→ →← S´R M V	→ T G
Increase molarity	← ← H A S´R S	← PECys P
Decrease pH	→ DTT D	→ G E

PROCISE C Chromatography

Passing Chromatogram Profile

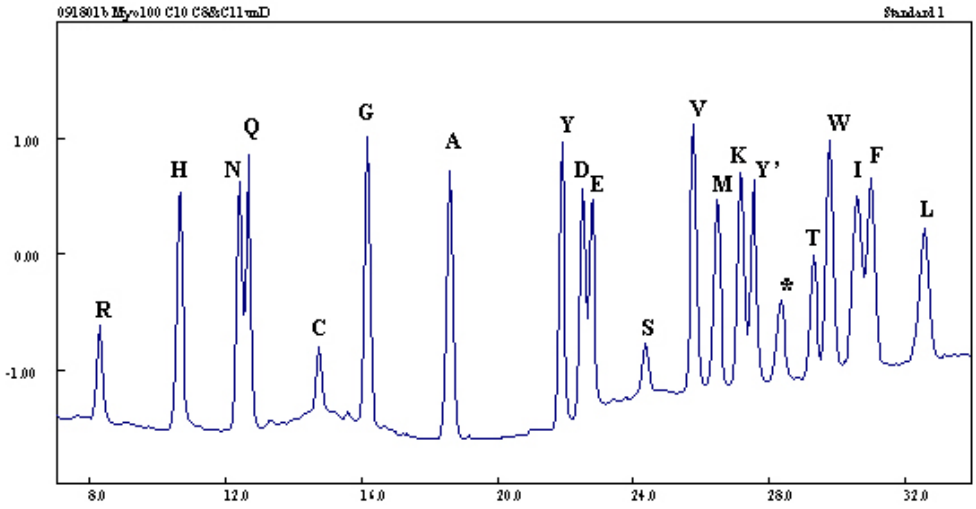


Figure 4-6 Twenty picomoles of ATH-Amino Acid Standard at 4 mAUFS. The separation shown was run with 3.5% THF, 75 mM sodium acetate, 2 mM tetrabutylammonium hydroxide as solvent A. Using tetrabutylammonium hydroxide in place of DIEA as the solvent A additive reverses the elution positions of H and R.

ATH-Amino Acid Elution Order

The following list identifies the ATH-amino acid derivatives in their expected elution order and identifies any chemical modifications.

ATH-Amino Acid Derivative Identity		
R	Arginine	(unmodified)
H	Histidine	(unmodified)
N	Asparagine	(unmodified)
Q	Glutamine	(unmodified)
C	Cysteine	(S-propionamide)
G	Glycine	(unmodified)
A	Alanine	(unmodified)
Y	Tyrosine	(unmodified)
D	Aspartic Acid	(piperidine amide)
E	Glutamic Acid	(piperidine amide)
S (and C)	Dehydroalanine	

ATH-Amino Acid Derivative Identity		
V	Valine	(unmodified)
M	Methionine	(unmodified)
K	Lysine	(e-phenylurea)
Y’	Tyrosine	(O-acetyl)
*	Naphthylmethylthiocyanate	
T	Threonine	(dehydro-)
W	Tryptophan	(unmodified)
I	Isoleucine	(unmodified)
F	Phenylalanine	(unmodified)
L	Leucine	(unmodified)

C-Terminal Gradient Conditions

The typical C-terminal gradient conditions are:

Time	%B	Flow Rate
0.0	15	300
10	27	300
20	37	300
32	42	300
32.1	90	300
37	90	300
37.1	90	50
50	90	10

Column temperature: 45 °C.

Flattening the Baseline

To achieve a high-sensitivity sequence, baseline rise must be kept to a minimum. One factor that causes baseline rise in the chromatograms is the slightly higher absorbance properties of solvent B2. Eliminating this factor increases the accuracy of chromatographic peak detection and integration by the SequencePro™ Data Analysis Application software, especially at high sensitivity.

Acetone has a very high UV absorbance at 269 nm—an optimal wavelength for PTH amino acid analysis. When small amounts of acetone are added to solvent A3, and a linear gradient is used, the absorbance of solvents A3 and B2 more closely match. The addition of acetone eliminate most of the baseline rise.

Adding Acetone to Solvent A3

WARNING **CHEMICAL HAZARD**. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **Solvent A3 (3.5% Tetrahydrofuran (THF))** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and liver and kidney damage. It may form explosive peroxides. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To add acetone to solvent A3:

1. Make a 1% acetone/H_2O solution by mixing 1 mL of HPLC-grade acetone with 99 mL of deionized water in a 100-mL clean bottle.
2. Add 700 µL of the acetone/deionized water solution to 450 mL of solvent A3 and mix well.

   Note: Additional acetone may be required to flatten the baseline.

   Add acetone in increments of 50 µL each until satisfactory results are obtained. Once the proper volume has been determined for a particular system, the volume should not change significantly.

Retention Time Reproducibility for Arginine and Histidine

The histidine-ATH and arginine-ATH derivatives may elute in different positions in a residue cycle than they do in the ATH-standard cycle using the standard buffer formulation. This problem can be minimized by preparing an alternative formulation of solvent A3. This formulation requires the purchase of tetrabutylammonium hydroxide solution; the 1M solution available from Fluka is a suitable product (Fluka P/N 86832).

WARNING **CHEMICAL HAZARD**. **Solvent A3 (3.5% Tetrahydrofuran (THF))** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and liver and kidney damage. It may form explosive peroxides. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **3M Sodium Acetate buffer,** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare an alternative formulation of solvent A3:

1. Open a new 1L bottle of solvent A3.
2. Add 25 mL of 3M Sodium Acetate Buffer, pH 5.5
3. Add sufficient tetrabutylammonium hydroxide solution to give a final concentration of 2 mM.
4. Add 5 mL of 1% acetone in water.
5. Cap the bottle and mix well.

Optimizing the Separation of the ATH Derivatives

To Improve the Separation of...	Action	Negative Effects
N/Q	Reduce initial %B	May reduce resolution in the later half of the chromatogram
D/E	Increase column temperature	Reduces separation of F/I
T/W	Increase initial %B	Reduces separation of N/Q
F/I	Reduce %B at 20 minutes	Reduces separation of K/Y'

Amino Acids Identifiable by C-terminal Sequencing

Nineteen amino acid residues have identifiable ATH-derivatives. These amino acids are included in the ATH-amino acid standard:

Ala	Leu
Arg	Lys
Asn	Met
Asp	Phe
Cys	Ser
Gln,	Thr
Glu	Tyr
Gly	Trp
His	Val
Ile	–

Note: Proline residues stop sequencing. There is no ATH-derivative included in the standard mixture for Pro.

Amino Acids Modified Prior to or During C-Terminal Sequencing

Arg Most of the Arg is acetylated during the initial C-terminal activation. This derivative is identifiable by a unique retention time, but it has not been characterized and is not included in the ATH-AA standard.

Asp and Glu Both of these amino acids with side-chain carboxylic acid groups are modified to piperidine amides during the automated chemistry. This modification prevents the side-chain carboxyls from interfering in the sequencing chemistry and provides easily detectable derivatives for these amino acids. The piperidine amide derivatives of Asp and Glu are included in the ATH-AA standard.

Cys Cys residues must be reduced and alkylated before sequencing for unambiguous identification. Acrylamide alkylation provides a Cys derivative with good stability to the sequencing chemistry and good chromatographic positioning. Without prior modification, Cys residues are detected as dehydroalanine, and as such are indistinguishable from Ser residues. The acrylamide derivative of Cys is included in the ATH-AA standard.

Gly (at the C-terminus) Gly, at the C-terminus of the protein, is detected in unacetylated and acetylated forms. Internal Gly residues are detected only in unacetylated form. Acetylated Gly is not included in the ATH-AA standard.

His Some His is recovered in unmodified form during sequencing. However some His is acetylated during the initial C-terminal activation or modified in other ways. These derivatives are not characterized.

Lys The standard protocol is to manually pretreat samples with phenylisocyanate which yields e-phenylcarbamyl-Lys. This Lys derivative is included in the ATH-AA standard.

Ser and Thr During the initial activation, Ser and Thr are acetylated and subsequently undergo ß-elimination, yielding dehydroalanine from Ser and the corresponding dehydro-analog from Thr. The ATH standards of Ser and Thr also undergo ß-elimination during preparation for chromatography and are detected as the dehydro-analogs.

Tyr Tyr is detected in unacetylated and acetylated forms. Both Tyr and acetylated-Tyr are included in the ATH-AA standard.

Expected C-Terminal Sequencing Results

Yields

A key point to be realized is that Pro residues stop sequencing. Callable sequence stops at the residue before Pro. In general, as opposed to N-terminal sequencing, C-terminal sequencing performance is sequence dependent. Initial yield and sequencing efficiency vary according to the specific amino acid sequence being analyzed.

Typical Initial Yields	If...
15-30%	Ala, Asn, Gln, Gly, Ile, Leu, Lys, Met, Phe or Val is at the C-terminus.
5-15%	Arg, Cys, Glu, His, Thr or Tyr is at the C-terminus.
<5%	Asp or Ser is at the C-terminus.

What is the effect of either Asp or Glu at the initial C-terminus of the protein?

Asp and Glu, during initial activation, can also form the respective symmetric anhydrides between the side-chain and alpha-carboxylic acid groups. This side-reaction contributes to a significant decrease in the initial yields, particularly for Asp.

What are the effects of specific residues on the efficiency of C-terminal sequencing?

Residues that...	Are...
do not reduce sequencing efficiency	Ala, Asn, Cys, Gln, Gly, Ile, Leu, Lys, Met, Phe, Tyr, Trp and Val.
may slightly reduce sequencing efficiency	Arg, Glu, His and Thr.
may reduce sequencing efficiency	Asp and Ser.
may be impossible to unambiguously identify and may reduce the sequencing yield below callable levels	consecutive Ser and/or Thr.

Typical Apomyoglobin Cycle 1 Results

The chromatogram examples of Apomyoglobin cycle 1 (Glycine) below are at 50 mAUFS and taken from two different sequencers. They show Glycine residue from 1 nmol of horse apomyoglobin applied to a ProSorb® filter. The methylimidazole catalyzed acetylation of Ser and Thr hydroxyls also causes acetylation of Gly (G') at the C-terminus.

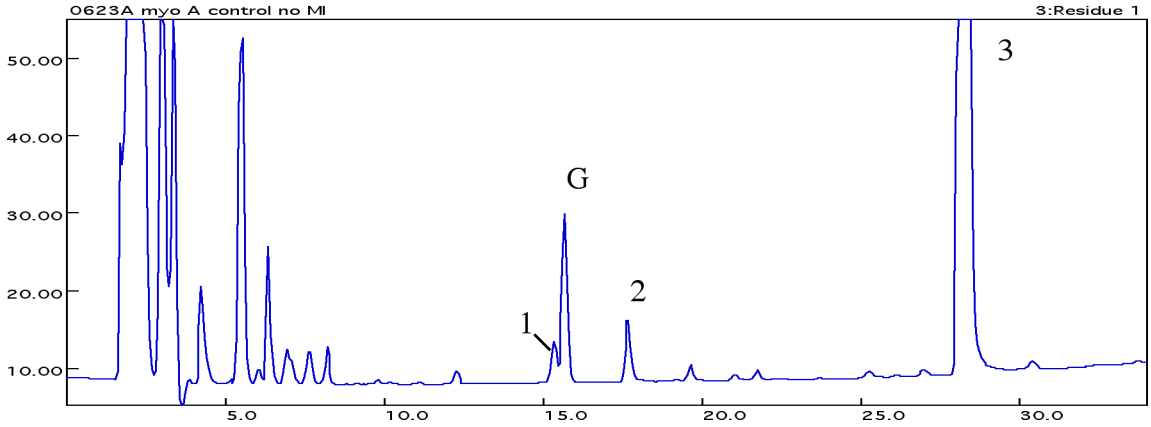


Figure 4-7 Cycle 1: Apomyoglobin (1nmol), no OH capping

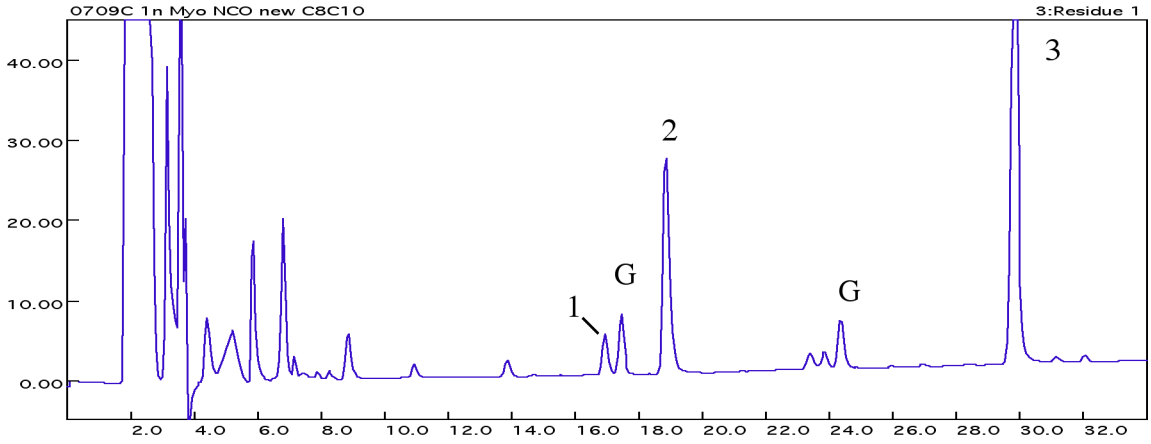


Figure 4-8 Cycle 1: Apomyoglobin (1 nmol), with OH capping

Typical Results for Apomyoglobin Yield

Yield	%
Initial	15 - 30
Repetitive	65 - 80%

Major Chemistry Artifact Peaks

Peak #	Location	Chemical
1	Peak before Gly at 5 - 15 mAU	not chemically identified
2	Peak before Ala at 5 - 40 mAU	not chemically identified
3	Peak at 80 - 240 mAU	Naphthylmethylthiocyanate, a reaction product of bromomethylnaphthalene and tetrabutylammonium thiocyanate

Custom Functions, Cycles, Methods, and Gradients

5

This chapter describes how to modify and create functions, cycles, sequencing methods, and gradient programs.

Refer to Appendix C, “Valves, Functions, Cycles, and Methods,” for more information about standard functions, cycles, sequencing methods, and gradient programs supplied with this system.

The following topics are contained in this chapter:

Creating and Modifying Functions

Guidelines

Use the following guidelines and procedures to create and modify functions.

- Functions 401–450 are allocated for user-defined functions.
- The standard functions included with the PROCISE Protein Sequencing Systems cannot be:
  - Directly modified.
  - Saved under a different name and then modified. You must create a new function using one of the function numbers reserved for user-defined functions (401–450).
- Only functions created by users can be directly modified.

The number of valves that can be activated per function is limited:

Valves	Maximum Valves Activated per Function
1–23 34–40 45, 46, and 63	8 valves can be activated per function
24–33 41–44 47–62	6 valves can be activated per function

Note: A maximum of eight valves can be activated simultaneously.

Creating a New Function

Note: Functions cannot be created or modified while the protein sequencer is in use.

To create a function:

1. Select the **Functions** dialog box from the dialog box pop-up menu.
2. Select a User Function (401 to 450) from the function list.
3. Highlight the Function Name field and enter a unique function name.
4. Move the cursor to the Valves Activated field.
5. Enter the valve numbers to be activated for the function. Enter a space between each valve number.
6. Repeat steps 2–5 for all the functions you want to create or modify.
7. Pull down the File menu from the main menu bar. Select **Save Function** to save the new or modified function.

Modifying a Function

To modify a function:

1. Select the Functions dialog box from the dialog box pop-up menu.
2. Select a user function (401 to 450) from the function list.
3. Highlight the Function Name field, and enter a unique function name.
4. Move the cursor to the Valves Activated field.

5. Enter the valve numbers to be activated for the function. Enter a space between each valve number.
6. Repeat steps 2– 5 for all the functions you want to create or modify.
7. Pull down the File menu from the main menu bar. Select **Save Function** to save the new or modified function.

Setting and Activating a Global Time

Global Time Overview

Each function is activated for a specific period of time. This period can be modified, either locally or globally.

When the global value of a function is changed from the Functions dialog box, the duration of that particular function is automatically changed in every cycle it is used.

The standard cycles included with this system use global time values for the *Load X1* cartridge function, and the flask functions Pre-Conversion Dry, Post-Conversion Dry, and Concentrate Sample only.

Functions that can be run with a global time setting are identified with a check mark under Global. When the function is highlighted, "yes" appears in the Global box.

A check mark in the Global box for a function used within a cycle or procedure indicates the global time value set from the Functions dialog box is being used for that function.

Global Times for Functions

Global times for a function can be set by modifying the function from the Functions dialog box.

A global time for a function cannot be directly activated in the standard cycles and procedures provided with this system. Instead, you must save a standard cycle or procedure under a new name, or create a new cycle or procedure that includes the modified function.

Setting a Global Time

- The protein sequencer must be idle to set a global time.
- Global times are set from the Functions dialog box.

To set the global time:

1. Select the Functions dialog box from the dialog box pop-up menu.
2. Highlight the function from the function list.
3. Enter the desired global time in the Global Value box.
4. Open the File menu, and select Save Function.

Activating a Global Time

The first step in activating a global time is to create a custom cycle or procedure.

To create a custom cycle or procedure:

1. Select the Cycles & Procedures dialog box from the dialog box pop-up menu.
2. Select the appropriate type of cycle or procedure from the cycle and procedure type pop-up menu.

3. Select the specific cycle or procedure you wish to include the modified function in from the cycle and procedure pop-up menu.
4. Open the File menu, and select **Save Cycle/Procedure As**.
5. Enter a unique name for the cycle or procedure.

Include the Modified Function

To include the modified function in the custom cycle/procedure:

1. Select the step (function) with the global time to be activated.
2. Click the Global box to activate the global time. An X appears in the box, a check mark in the Global column, and the global value in the Value column.
3. Open the File menu, and select Save Cycle/Procedure.

Create a Custom Method

To create a custom sequencing method:

1. Open the Sequence Methods dialog box, and select the method you wish to use as a template from the Method pop-up menu.
2. Open the File menu, and select **Save Method as**.
3. Enter a unique name for the new method.
4. Open the appropriate cycle pop-up menu, and select the cycle or gradient you have modified.
5. Open the File menu, and select **Save Method**.

Modifying Cycles

General Guidelines

- The standard cartridge and flask cycles included with this system cannot be modified directly. You must create a custom cycle by:
  - Saving an existing cycle under a new name
  - Editing the cycle
  - Saving the changes under the new cycle name
- The maximum number of steps allowed per cycle is 300.
- Every cycle requires a Begin and an End step.

Guidelines For Cartridge Cycles

- The Ready to Transfer step in a Cartridge cycle synchronizes with the Ready to Receive step in a Flask Cycle.
- The cartridge cycle must have Ready to Transfer and Transfer Complete steps to transfer sample from the reaction cartridge to the flask.

Guidelines For Flask Cycles

- The Ready to Receive step in a Flask cycle synchronizes with the Ready to Transfer step in a Cartridge Cycle.
- The flask cycle must have a Ready to Receive step to receive sample from the cartridge.
- The Prepare Pump step starts the pump and instructs it to equilibrate the column at the initial conditions specified for the start of the gradient. Allow at least 17 min between the Prepare Pump and Load Injector steps.

Modifying a Cycle

To modify a standard flask cycle:

1. Select the **Cycles & Procedures** dialog box from the dialog box pop-up menu.
2. Select the cycle or procedure type from the Cycle and Procedure Type pop-up menu.
3. Select the cycle or procedure from the Cycle and Procedure pop-up menu.
4. Pull down the File menu from the main menu bar. Select **Save Cycle/Procedure As.**
5. Type the new cycle name, and click **OK**.

If you need to...	Then...
delete a row	1. Highlight the row to be deleted. 2. Click **Delete Row**.
insert a row	1. Select the function to be inserted from the function list. The function can be selected by using the scroll bar or by typing the function number at the top-right hand corner of the function list. 2. Highlight the row immediately before the insertion point, and click Insert. 3. To enter the function run time, click the Global box to turn the global time off. 4. Type the function time in seconds in the Value box.

6. Pull down the File menu from the main menu bar.
7. Select **Save Cycle/Procedures** to save your changes.

Creating Cycles

General Guidelines

Follow the general guidelines below for creating cycles:

- The maximum number of steps allowed per cycle is 300.
- A cartridge cycle must include a Ready to Transfer and Transfer Complete step. The Ready to Transfer step in a Cartridge cycle synchronizes with the Ready to Receive step in a Flask Cycle.
- A flask cycle must include a Ready to Receive step to receive sample from the cartridge. The Ready to Receive step synchronizes with the Ready to Transfer step in a Cartridge Cycle.
- The Prepare Pump step starts the pump and instructs it to equilibrate the column at the initial conditions specified for the start of the gradient. Allow at least 17 min between the Prepare Pump and Load Injector steps.
- Every cycle must have a Begin and an End step.

Creating a Cycle

To create a cycle:

1. Select the **Cycles and Procedures** dialog box from the dialog box pop-up menu.
2. Select the cycle type from the cycle and procedure type pop-up menu.
3. Select **User Defined Cycle 1** from the cycle and procedure pop-up menu.

If you need to...	Then...
delete a row	1. Highlight the row to be deleted. 2. Click **Delete Row**.
insert a row	1. Select the function to be inserted from the function list. The function can be selected by using the scroll bar or by typing the function number at the top-right hand corner of the function list. 2. Highlight the row immediately before the insertion point, and click Insert. 3. To enter the function run time, click the **Global box** to turn the global time off. 4. Type the function time in seconds in the Value box.

4. Pull down the File menu from the main menu bar.
5. Select S**ave Cycle/Procedures As**.
6. Type the new cycle name, and click **OK**.

Modifying Methods

Guidelines

The standard methods included with this system cannot be modified directly.

To modify a standard method, you must:

- Save an existing method under a new name
- Edit that method
- Save your changes under the new name

Note: Nine exception cycles are allowed per method.

Modifying a Method

To modify a standard method:

1. Select the Sequence Methods dialog box from the dialog box pop-up menu.
2. Select the method to be copied from the Method pop-up menu.
3. Pull down the File menu from the main menu bar, and select **Save Method As**.
4. Type the new method name, and click **OK**.
5. Highlight the default method row.
6. Select the new cartridge cycle, flask cycle, and/or gradient from each pop-up menu.

If you need to...	Then...
delete a row	1. Highlight the row to be deleted. 2. Click **Delete Row**.
insert a row	1. Highlight the row after which the new row will be inserted. 2. Click **Insert Row**. 3. Move the cursor to the cycle # field, and enter the cycle number to be added as an exception. 4. Select the new cartridge cycle, flask cycle, and/or gradient from each pop-up menu.

7. If the cartridge, flask, or column temperatures need to be changed:
   a. Move the cursor to the appropriate temperature field
   b. Enter the desired temperature.
8. Pull down the File menu from the main menu bar, and select **Save Method**.

Creating Methods

General Guidelines

A method must contain a valid default cycle (the cycle that is run when there is no exception cycle). The default cycle is not necessarily a standard cycle.

Note: Nine exception cycles are allowed per method.

Creating a Method

To create a method:

1. Select the Sequence Methods dialog box from the dialog box pop-up menu.
2. Select the **User Defined** method from the Method pop-up menu.
3. Highlight the default method row.
4. Select the new cartridge cycle, flask cycle, and gradient from each pop-up menu.

If you need to...	Then...
delete a row	1. Highlight the row to be deleted. 2. Click **Delete Row**.
insert a row	1. Highlight the row after which the new row will be inserted. 2. Click I**nsert Row**. 3. Move the cursor to the cycle # field, and enter the cycle number to be added as an exception. 4. Select the proper cartridge cycle, flask cycle, and gradient for the cycle from each pop-up menu. 5. Enter the desired cartridge, flask, and column starting temperatures.

5. Pull down the File menu from the main menu bar, and select **Save Method As**.
6. Type the new name, and click **OK**.

Overview of Gradient Programming

General

- Routine operation of the HPLC components of the system is controlled by the PROCISE control software.
- Solvent gradient programming changes the retention time of sample species automatically during the course of a single chromatographic run.
- Both gradient programs and changes to the composition (ionic strength) of solvent A3 are used to optimize the retention times of the PTH-amino acids.
- The standard gradient program, Prepare Pump cLC, is used in sequencing methods to prepare the pump, UV Detector and column for a run.
- One standard, analytical gradient program—Normal 1 cLC—is included with this system. The gradient conditions for Normal 1 cLC are listed in Table 5-1.

Note: For more detailed information on menus used to control the 140D pump, refer to the *ABI 140D Microgradient Delivery System User's Manual (P/N 903586*.)

Table 5-1 Gradient conditions for Normal 1 cLC

Target Pressure:		1500 psi				
Target Time:		0.2 min				
Pressure Limits:		0 to 3500 psi				
Data Collection Time:		28 min				
Step #	**Time (min)**	**% B**	**Flow Rate (µL/min)**	**Events On**	**Volume Used AB**	
1	0.0	10	40	12	0	0
2	0.4	12	40	1	14	1
3	4.0	22	40	1	133	25
4	22.0	50	40	1	593	284
5	22.6	90	40	1	600	300
6	23.5	90	40	1	603	332
7	29.0	90	60	0	636	629
8	33.0	50	20	0	660	685

Phases of a Gradient Program

A typical program-controlled gradient run consists of three phases:

- Prepressurization
- Equilibration
- Gradient

Prepressurization Phase

During prepressurization, the 140 pump rapidly pressurizes to a set of initial conditions specified by the program. Then, the pump ramps to the conditions desired for equilibration and the first step of the gradient phase—*time-zero* conditions.

Equilibration Phase

During equilibration, the time-zero conditions are held for a specific period of time to allow the system to achieve a steady state before beginning the gradient.

Gradient Phase

The gradient phase (gradient) is started either by sample injection, or by Function 232, Start Gradient. Sample separation occurs during the gradient, which consists of a variable number of steps. Each step is characterized by a specific duration, flow rate and mobile phase (solvent) composition. Typically, the composition is gradually changed as a linear function of time from step to step.

External Events

- External events are activated and deactivated by relays located on the back of the 140 pump (events 1 through 4 on the pump terminal block).
- Controlled by programmed runs, events include integrator start, detector autozero, chart recorder start/stop, additional A/D start/stop, or additional data collection start/stop.
- Data collection by the SequencePro™ Data Analysis Application software starts automatically when sample injection occurs.
- Relays are activated (closed) when you select 1, 2, 3, and/or 4 in the Event column of your gradient program.
- Events remain active until the corresponding numbers are removed from the Event column, or until the pump receives an End of Run signal.
- The End of Run signal deactivates all external events by opening all the relays.

Creating and Modifying Gradient Programs

Gradient Program Parameter Overview

The key parameters of a typical gradient program for the Procise cLC Protein Sequencing System are shown in Table 5-2.

Table 5-2 Key parameters of a typical gradient program

Max Pressure	Maximum operating pressure for the system. If the system pressure rises above this value, operation of the 140 pump is halted. Choices are 0 through 4000. The default value is 4000. Select an upper pressure limit compatible with your column. As a general rule, set the maximum operating pressure 1000 psi above the expected operating pressure of the system.
Min Pressure	Minimum operating pressure for the system. A pressure below the specified value will halt operation of the 140 pump. Typical values are 0 to 100 psi. The default value is 0.
Target Pressure	The pressure the 140 pump is programmed to reach during the first part of the pre pressurization phase. Choices are 0 to 4000 psi. Typical values are 1500 psi at a flow rate of 40 µL/min. The default value is 1650 psi. Generally, the target pressure should be roughly equal to the expected back pressure of the system at the start of the gradient phase.
Target Time	The amount of time the 140 pump will take to ramp from the target pressure to the pressure desired for the first step in the gradient. Choices are 0.1 to 99. Typical values are 0.2 to 1.0 min for gradients starting with a composition greater than 10% B and a flow rate greater than 50 µL/min. Target times as long as 10 min may be required for gradients starting with a 0% B composition and/or a low flow rate such as 10 µL/min. The default value is 0.2 min.
Equilibrate Time	A typical equilibration time for the Procise cLC Protein Sequencing System is 18 min.
Data collection time	The length of time data is collected by the PROCISE control software. Sample injection initiates data collection. The default value is 28 min.

Gradient Phase of Run

Table 5-3 describes the steps in the gradient phase of a typical programmed run. The gradient program, Normal 1 cLC, is used for this example. Customized programs are created by modifying an existing program, and saving it under a new name.

Table 5-3 Typical steps of the gradient phase of a programmed run

Step	Time[1]	%B[2]	Flow Rate (µL/min)	Events C=closed O=open	Description
1	0.0	12	40	1–C 2–C	The 140 pump begins pumping at 12% B. Combined flow from pumps A and B is 40 µL/min. Selecting C for event 1 turns the chart recorder on. Selecting C for event 2 auto-zeroes the detector.
2	0.4	12	40	1–C	This short hold at 12% B allows for good resolution of peaks S through G. The autozero from step 1 is released by deselecting event 2.
3	4.0	22	40	1–C	From time 0.4 to time 4.0, the 140 pump linearly increases from 12% B to 22% B.
4	22.0	50	40	1–C	From time 4.0 to time 22.0 (18 min), the 140 pump linearly increases the % B from 22 to 50%.
5	22.6	90	40	1–C	The 140 pump linearly increases the % B from 50 to 90%. Combined flow from pumps A and B remains constant at 40 µL/min.
6	23.5	90	40	1–C	Flow and composition remain the same for 1.5 min. Contaminants and by-products are removed from the column to clean it for the next sample.
7	29.0	90	60	1–O	Selecting O for event 1 turns the chart recorder off.
8	33.0	50	20		The 140 pump stops flow to the column 33 min after injection, unless it receives another PREPARE PUMP message. The pump can be programmed to continue running indefinitely after injection until it receives a PREPARE PUMP message. If you choose this option, we recommend that you let the pump run at 20 µL/min, 50% B.

1.The smallest time increment for any gradient program step is 0.1 min.
2.The % B values are suggested starting values and may need adjustment to resolve all amino acid peaks. See Chapter 4, "Optimizing Sequencer Processes," for more information on adjusting the gradient and solvent composition to correct poor resolution of PTH-amino acids.

Gradient Programming Guidelines

- The standard gradient programs provided with this system can be modified.
- During a run, the active gradient can be modified. However, the changes you make will effect future cycles only, not the current cycle.
- You can create custom gradient programs by using an existing program as a template, and saving it under a new name.

Pump Control

Functions

Two pump control functions are included with the system:

- Function 227, Prepare Pump
- Function 232, Start Gradient

Definitions and Guidelines

Function 227, Prepare Pump, halts the pump, refills the syringes, pressurizes the pump, and then runs the pump at the time zero conditions specified in the gradient program.

- Prepare Pump also downloads the gradient program to the pump. Changes made to a gradient program on the computer will not take effect until the next time the gradient is downloaded from the computer as part of Function 227.

  Function 232, Start Gradient, starts the gradient phase of a program without an injection.
- When programming a cycle, Function 227, Prepare Pump, must occur **at least 18 min** before the sample is injected on the column. This allows for column equilibration at the time zero conditions defined in the program.
- The smallest time increment for any gradient program step is 0.1 min.

  IMPORTANT! Inadequate equilibration will result in variable retention times and resolution.
- The time of the last step in a program is the End of Run time. After the last step, the pump stops flow to the column, and waits for the next Prepare Pump message. When the pump receives the next Prepare Pump message, it refills, and automatically begins the specified programmed gradient run.

Note: After the last step in the program, the pump stops flow to the column. In the gradient program, Normal 1 cLC, the pump stops 33 min after injection unless it receives another Prepare Pump message from the computer. The pump can also be programmed to continue running after the final step of a program. We **strongly recommend** that you configure the pump to continue running after the end of a gradient program. Refer to the 140D pump user's manual for more information.

When the Sample is in the Column

Once the sample is injected onto the column, continue the solvent flow until all sample components elute from the column and pass through the detector. Sample components remaining on the column may elute during a subsequent run, and interfere with peak identification and quantitation.

- A flow rate and % B must be specified in each step of the gradient phase of a program.
- If a new flow rate is not specified for each step, the value from the previous step is used.
- If a % B value is not specified for each step, the default value of 0% B is used.

Time Between Steps

The time between the Prepare Pump and Load Injector steps must be limited because the syringes in the 140 pump have a limited volume:

- Time limit depends upon the time-zero flow rate and % B conditions, and the volume of solvents required during the pressurization and analysis cycles.
- If the limit is exceeded, the pump continues pumping until one of the syringes empties, or until the start signal arrives.
- If the start signal is received late (because of a hold or pause in the protein sequencer cycle), there may not be enough buffer in the syringes to complete the analysis.
- When the syringes empty, the analysis terminates and the syringes refill in preparation for the next run before elution is complete.
- Sample components remaining on the column may elute during a subsequent cycle and interfere with peak identification and quantitation.

Creating or Modifying Gradient Programs

To create or modify the gradient program:

1. Select the Gradient screen. Then select the program you wish to modify from the drop-down list.
2. Highlight the step to change.
3. Modify the values in the Time, % B, Flow Rate, and Events boxes as appropriate.
4. To insert or delete a row, highlight the Time line and click Insert Row or Delete Row as appropriate. Alter the Time, % B, Flow Rate, and Events as desired.
5. To save your changes, pull down the File menu from the top menu bar, and select either **Save Gradient** or **Save As**.

   When Save Gradient is selected, the original gradient program you selected in step 1 is modified.

   Note: Changes made to this program will not take effect until the next time the gradient program is used.

   When Save As is selected, you must enter a new, unique name for the modified gradient. The original gradient program you selected in step 1 is not modified.

Tests and Procedures 6

This chapter describes how to access the PROCISE® PC V2.0 software to run test procedures for the PROCISE® Protein Sequencing Systems.

The following topics are contained in this chapter:

Procedures Used for Startup, Shutdown, and Normal Operation

Guidelines to Perform Tests and Procedures

General Guidelines

Follow the guidelines below when performing tests and procedures.

- Run tests and procedures only while the protein sequencer is idle.
- Always allow tests and procedures to finish without interruption. Some tests and procedures change the protein sequencer's pressure settings.
- Look for tests and procedure results in the event log.

If a Test or Procedure Is Interrupted

If you must interrupt a test or procedure:

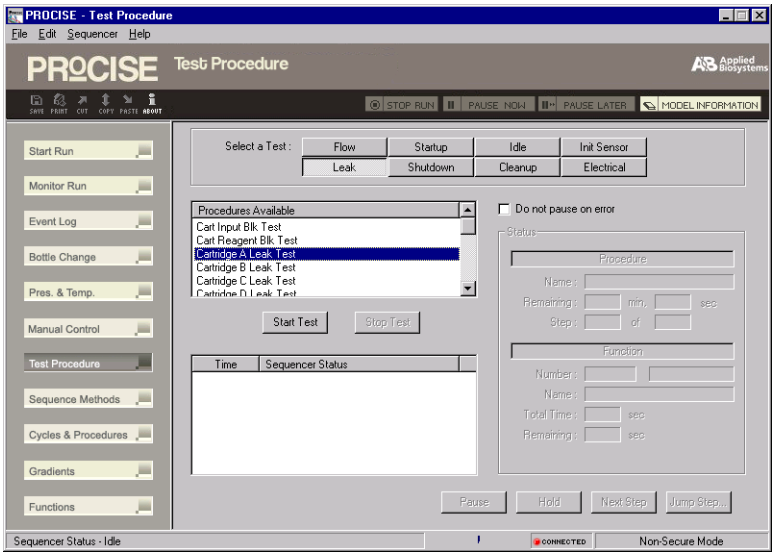
- Click **Next Ste**p repeatedly until the test finishes.
- Reset the default pressures and temperatures on the protein sequencer. Instructions are listed in "Restoring Default Pressures and Temperatures" on page 6-3.
- You can set up tests and procedures to pause whenever an error occurs, or you can elect to have the test/procedure run to completion without pausing for errors.

Don't Pause On Error Settings

While running a test or procedure, you can either select or deselect the Don't Pause On Error box in the Test dialog box.

If...	Then...
you deselect the Don't Pause on Error box	• the box is empty by default. • the test or procedure pauses when an error occurs. • a dialog box noting the error appears on the screen. • the tests being run sequentially are also paused. • you must click **Resume Test** after each error to continue the test or procedure.

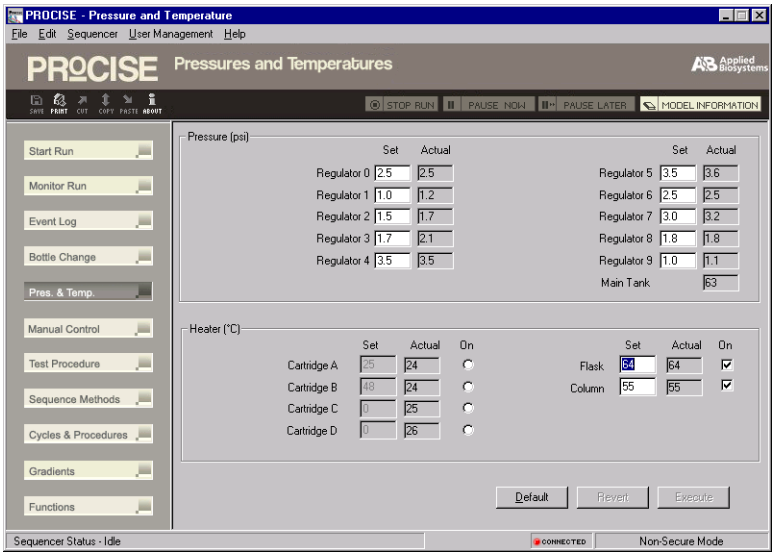
If...	Then...
you select the Don't Pause On Error box	the box has an X in it (figure below).
	The test or procedure will run to completion without pausing if an error occurs.



Restoring Default Pressures and Temperatures

To restore default pressures and temperatures:

1. Select the Pressures & Temperatures dialog box from the PROCISE main menu.



2. Click **Default** to restore the default pressures and temperatures recommended by Applied Biosystems.

Note: Under certain conditions, pressure settings other than the default settings may be desired.

Startup and Shutdown Procedures

Startup Procedure

One startup procedure, Startup Procedure, is included with the PROCISE control software. The Startup Procedure:

- Flushes each reagent/solvent bottle with argon
- Refreshes the reagent in the delivery line
- Washes the flask with S4

Note: No solvent or reagent is delivered through the cartridges.

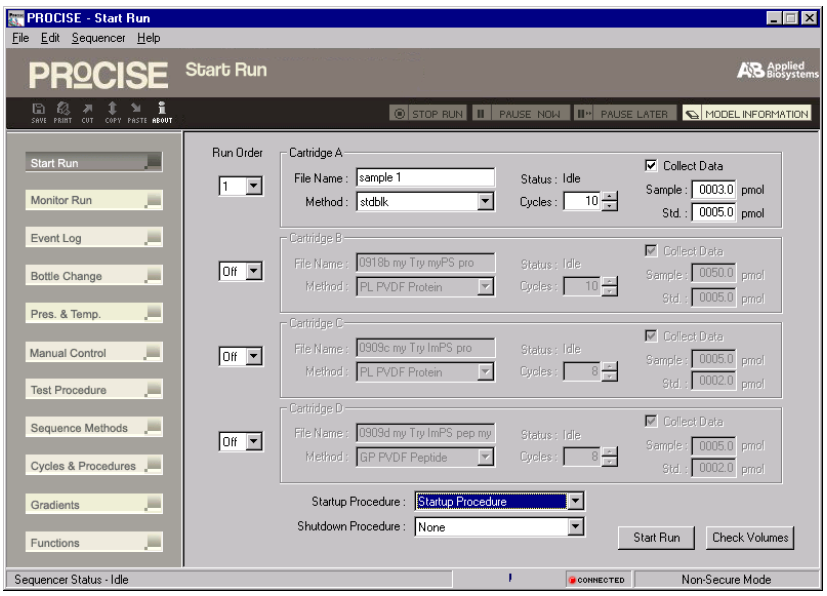
The Startup Procedure can be included as part of a sequencing run, or it can be run independently from the Test dialog box.

Startup Procedure as Part of a Sequencing Run

To run the Startup Procedure:

1. Select the Start Run dialog box from the PROCISE Main menu.

   The Startup and Shutdown procedures pop-up menus are at the bottom of the screen.



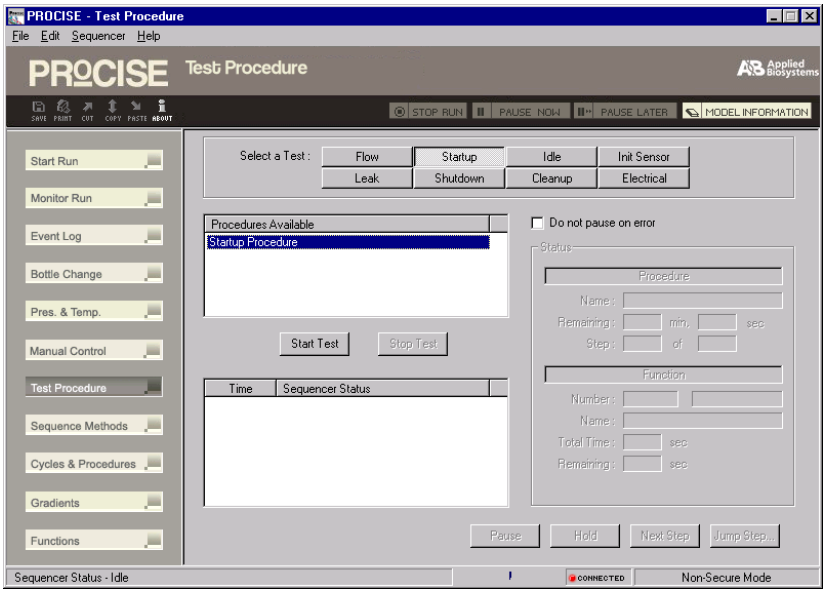
2. Select **Startup Procedure** from the Startup pop-up menu.
3. Cleanup procedure(s), (page 6-23), can be executed after completion of the last cartridge scheduled to run.

Note: When included as part of a run, the Startup procedure is executed immediately after sensor initialization.

Running the Startup Procedure Independently

To run the Startup Procedure independently, using the Test dialog box:

1. Select the Test dialog box from the PROCISE Main menu.



2. Click **Startup**, and select **Startup Procedure**.
3. Select or deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
4. Click **Start Test** to run the procedure.
5. Allow the procedure to run to completion.

Overviews of Shutdown Procedures

Two shutdown procedures are included with the PROCISE control software:

- The Post-Run Valve Block Wash X1-X2
- The Short-Term Shutdown procedure

Post-Run Valve Block Wash Overview

The Post-Run Valve Block Wash washes the system flow paths:

- From the X1 bottle position with methanol
- From the X2 bottle position with acetonitrile

Note: No solvent is delivered through the reaction cartridges or to other bottles.

Ways to Run the Post-Run Valve Block Wash

The Post-Run Valve Block Wash can be run two different ways:

- From the Start Run dialog box as part of a sequencing run. The procedure is executed at the end of the sequencing run
- From the Test dialog box as an independent step

IMPORTANT! Before starting this procedure, make sure methanol is loaded in the X1 bottle position, and acetonitrile is loaded in the X2 bottle position.

WARNING **CHEMICAL HAZARD**. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Running the Post-Run Valve Block Wash

To run the Post-Run Valve Block Wash procedure as part of a sequencing run:

1. Select the Start Run dialog box from the dialog box pop-up menu. At the bottom of the dialog box are the Startup and Shutdown procedures pop-up menus.
2. Select **Post-Run Valve Block Wash X1-X**2 from the Shutdown pop-up menu.

To run the Post-Run Valve Block Wash procedure independently:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Shutdown**.
3. Select **Post-Run Valve Block Wash X1-X**2 from the test menu.
4. Select or deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
5. Click **Start Test**.
6. Allow the procedure to run to completion.

Short-Term Shutdown Procedure Overview

If the protein sequencer will be idle for 1 to 2 weeks, execute the Short-Term Shutdown procedure.

This procedure:

- Washes all the valve blocks, delivery lines, and loops with ethyl acetate (S2B)
- Washes the flask and injector with S4
- Backflushes each bottle with argon to remove reagents from the delivery line after common flow paths are washed and flushed with argon

Ways to Run the Short-Term Shutdown

The Short-Term Shutdown procedure can be run in two different ways:

- From the Start Run dialog box as part of a sequencing run.
  When included as part of a run, the procedure is executed after completion of the last cartridge scheduled to be run.
- From the Test dialog box as an independent step.

Running the Short-Term Shutdown Procedures

To include the Short-Term Shutdown procedure as part of a sequencing run:

1. Select the Start Run dialog box from the dialog box pop-up menu. At the bottom of the dialog box are pop-up menus for Startup and Shutdown procedures.
2. Select **Short-Term Shutdown** from the Shutdown pop-up menu.

To run the Short-Term Shutdown procedure independently:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Shutdown**.
3. Select **Short-Term Shutdown** from the test menu.
4. Select or deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
5. Click **Start Test**.
6. Allow the procedure to run to completion.

Initializing the Sensors

Overview

Depending on the number of cartridges, the protein sequencer has up to 11 optical sensors. The sensors are used to detect fluid deliveries.

Every time you click Start Run, the protein sequencer automatically runs the Init Sensor procedure that initializes the sensors. This procedure flushes the flow path through each sensor, then takes a dry reading for each sensor. If the sensor light path is not completely dry, the sensor will not function correctly during sequencing.

The Init Sensor procedure can also be run independently from the Test dialog box (the figure shown in step 2 of the procedure).

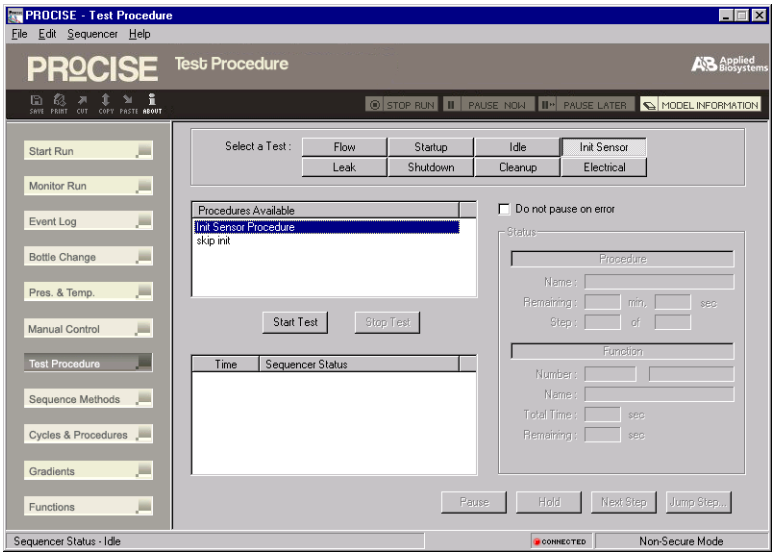
IMPORTANT! Always allow the Init Sensor procedure to run to completion. If the protein sequencer has been shut down, or if a sensor has been moved or replaced, the Init Sensor procedure must be run before sequencing or using manual control functions.

Init Sensor Procedure

To run the Init Sensor procedure:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Init Sensor**.

   The Init Sensor procedure is automatically selected (figure below).



3. Select or deselect the **Don't Pause On Error** box.

   Refer to page 6-2 for information on this option.

4. Click **Start Test**.

5. Allow the procedure to run to completion.

Bottle Change Procedure

Each bottle change procedure backflushes a specific chemical into the reagent bottle, then vents the bottle so you can change it.

CAUTION **CHEMICAL HAZARD. Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Bottle Change Procedure Stages

After the new bottle is loaded, the bottle change procedure:

- Flushes the bottle with argon gas
- Delivers the chemical to the waste bottle
- Washes the associated valve blocks and Teflon lines

Bottle Change Procedure Options

Two types of bottle change procedures are available for each bottle position.

- One allows you to change the selected bottle(s) only.
- The other procedure performs a leak test on the bottle position as well as a bottle change.

Note: The procedures that perform leak tests are identified by *-leak* at the end of the procedure name.

Note: The cycle time for each bottle change procedure is listed in Table 6-1, "Bottle change procedure list," on page 6-9.

Removing the Old Bottle

Note: The system must be idle or paused to run a Bottle Change procedure.

To remove the old bottle:

1. Select the Bottle Change dialog box from the dialog box pop-up menu. **Do not remove the bottle at this time.**
2. Select the appropriate bottle position, solvent, or column from the menu.
3. Select the appropriate procedure from the Bottle Change Procedure pop-up menu.
4. Place the cursor in the Chemical box and press the tab key on the computer keyboard. The cursor moves to the Lot Number box, and the lot number is highlighted.
5. Enter the lot number of the new bottle in the Lot Number box. The date is updated automatically.
6. Click **Change Bottle**.
7. When prompted, remove the old bottle and bottle seal.

Installing the New Bottle

To install the new bottle:

1. Install the new bottle.
2. Install a new seal on the rim of the new bottle.
3. Screw the new bottle into the bottle cap assembly, tightening until the seal contacts the top of the bottle cap assembly. Then turn the bottle approximately 1/4-turn more.

Avoid Racheting

IMPORTANT! Do not tighten bottles until a snapping sound (ratcheting) is produced by the bottle cap assembly. Ratcheting the bottle cap assembly causes premature wear and can crack the bottle seal.

Finishing the Bottle Change Procedure

To finish the Bottle Change procedure:

1. Click **Continue**. The procedure continues through the remaining steps, which includes priming the delivery line up to the valve block.
2. Repeat the procedure to change additional bottles if necessary.
3. When you are finished, pull down the File menu from the main menu bar.
4. Select **Save Chemicals**.

IMPORTANT! Leak tests use functions that alter protein sequencer operating pressures. If the procedure is aborted before completion, select the Pressures & Temperatures dialog box. Click **Default** to restore the default settings.

Table 6-1 Bottle change procedure list

Procedure	Cycle Time (min)
Bottle Change for R1	1:30
Bottle Change for R1 - leak	2:40

Table 6-1 Bottle change procedure list *(continued)*

Procedure	Cycle Time (min)
Bottle Change for R2	1:00
Bottle Change for R2 - leak	2:00
Bottle Change for R3	3:00
Bottle Change for R3 - leak	4:15
Bottle Change for R4A	1:35
Bottle Change for R4A - leak	3:00
Bottle Change for R5	1:30
Bottle Change for R5 - leak	2:55
Bottle Change for S1	1:30
Bottle Change for S1 - leak	2:55
Bottle Change for S2B	1:15
Bottle Change for S2B - leak	2:40
Bottle Change for S3	1:15
Bottle Change for S3 - leak	2:40
Bottle Change for S4C	1:15
Bottle Change for S4C - leak	2:40
Bottle Change for X1	2:40
Bottle Change for X1 - leak	4:05
Bottle Change for X2	1:55
Bottle Change for X2 - leak	3:20
Bottle Change for X3 (both)	4:20
Bottle Change for X3 (both) - leak	5:45

Idle Procedure

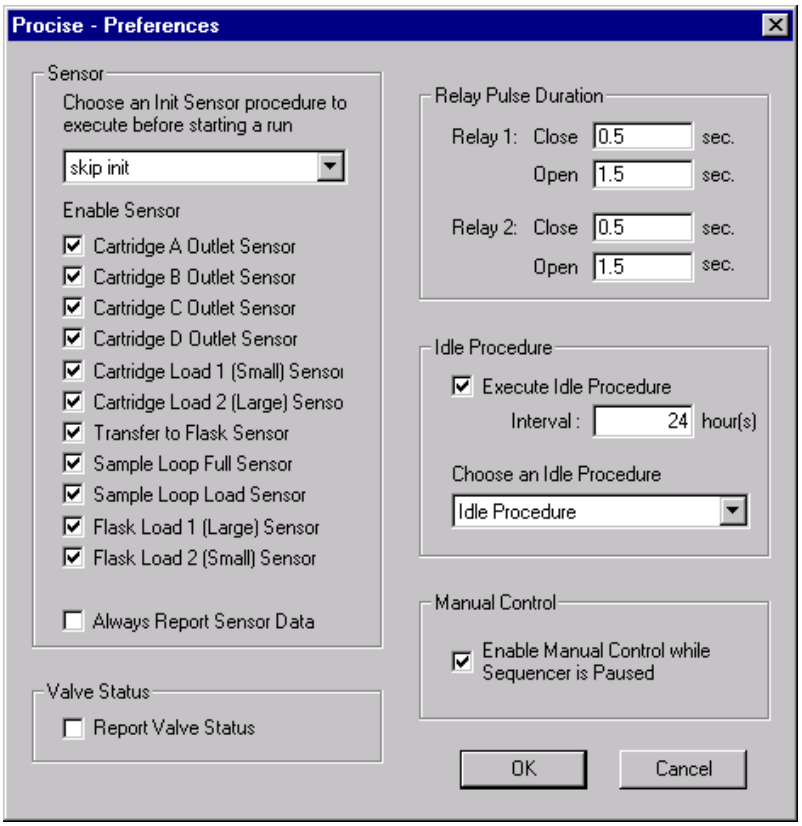
The Idle procedure minimizes sequencing problems due to chemical decomposition during inactive periods. When the protein sequencer is inactive, oxygen slowly diffuses into the system. The oxygen causes solvents and reagents to decompose and form by-products.

The Idle procedure flushes argon gas to each reagent and solvent bottle at a user-selectable level.

Running the Idle Procedure

To run the procedure:

1. Select Preferences from the Sequencer drop-down menu on the upper menu bar. The Preferences box (shown below) appears.



2. Select the box labeled Execute Idle Procedure.
3. Enter the frequency (in hours) that you want the procedure to run. Valid entries range from once every hour to once every 999 hours.
4. Click **OK**.

Note: If the protein sequencer is active when the Idle procedure is selected, go to the Start Run dialog box, and click Update.

Manual Injection Procedure

If you suspect that the flask chemistry is having a detrimental effect on sequencing results, use the manual injection procedure to inject the PTH-amino acid standard.

The following items are required for the manual injection procedure:

- Syringe with luer fitting
- 1/4-inch male to luer adapter (P/N 382-0007 in Spare parts kit 2)
- 1/4-inch female to 5/16 female union (P/N 403-0280 in Spare parts kit 2)

Preparing the PTH-Amino Acid Standard

To prepare the PTH-Amino Acid Standard for injection:

1. Prepare a 0.1 pmol / µL solution of PTH standard (5 pmol injected onto column)
2. Fill a clean measuring cylinder with approximately 5 mL of HPLC-grade water.
3. Add 10 µL of each PTH standard stock solution, including PMTC, to the measuring cylinder.
4. Bring to a total volume of 10 mL using HPLC-grade water.
5. Mix well.

Creating a Manual Injection Cycle

To create a Manual Injection cycle:

1. Scroll to the Cycles & Procedures dialog box, and select **Flask Cycl**e.
2. Select **Run Gradient** from the list of Flask cycle options.
3. Open the File from the top menu bar, and select **Save Cycle/Procedure as**.
4. Name the new cycle Manual Injection.
5. Click **OK**.

Modifying the Manual Injection Cycle

To modify the Manual Injection cycle:

1. Select **step 1, Begin**.
2. Select function 226, Load Position, from the function list.
3. Click **Insert Row**. The Global time will be assigned automatically.
4. Select what is now **step 6** (Wait).
5. Select function **223**, Inject Position, and click **Insert Row**.
6. Open the File menu from the top menu bar, and select **Save Cycle/Procedure** to save the cycle shown in Table 6-2.

Flask Cycle Information

Table 6-2 Manual injection flask cycle

Step	Function name	Function number	Time in sec	Global time	Elapsed time
1	Begin	258	0	—	:00
2	Load Position	226	0	—	:00
3	Wait	257	30	—	:30
4	Prepare Pump	227	1	4	:31
5	Wait	257	900	—	15:31
6	Wait	257	120	—	17:31
7	Inject Position	223	0	4	17:31
8	Start Gradient	232	1	—	17:32

Table 6-2 Manual injection flask cycle *(continued)*

Step	Function name	Function number	Time in sec	Global time	Elapsed time
9	Wait	257	900	—	32:32
10	Wait	257	780	—	45:32
11	End	259	0	—	45:32

Incorporating the Manual Injection Procedure

To incorporate the Manual Injection cycle into a method:

1. Scroll to the Sequence Methods dialog box, and select the **User Defined Method**.
2. For the Default line, choose **None** for the Cartridge Cycle, **Manual Injection** for the Flask Cycle, and **Normal 1** for the Gradient.
3. Change the cartridge starting temperature to 35 ˚C.
4. Open the File menu, and select **Save Method As**.
5. Name the method Manual Injection.

Performing the Manual Injection

To perform the manual injection:

1. Purge the 140 pump.
2. Remove the 5/16-inch fitting from port 42, and connect it to the coupler (white Teflon™ 5/16-inch to 1/4-inch union).
3. Install the 1/4-inch luer adapter.
4. From the Start Run dialog box, set up a run using Manual Injection as the Method. Set the number of cycles to 1, and click Start Run.
5. When the Init Sensor procedure starts, jump to the last step of the Init Sensor procedure.
6. If the column has reached the correct temperature (LED is cycling), click **Next Step** if the Flask cycle is at Waiting for Temperatures.
7. As soon as the Inject Position LED on the front panel display is set to OFF, connect the empty syringe to the luer fitting and flush out the sample loop.
8. Load the PTH amino acid standard solution into the syringe, and inject it manually into the sample loop.

Procedures Used for Periodic System Optimization

Flow Procedures

Flow Procedure Overview

The PROCISE Protein Sequencing System has six flow procedures. Only the Sensor & Delivery Test is normally performed by users. The other five tests are performed during instrument manufacturing and system installation.

The Gas Flows, Liq Del Test, Sensor Check, and Cart L2 Cal procedures are used during instrument manufacture only.

Stages of the Sensor & Delivery Test

The Sensor & Delivery Test verifies the operation of the fluid optical sensors in the protein sequencer.

The Sensor and Delivery Test consists of four stages:

Stage	Action
1.	Chemicals are delivered from the bottles or flask through specific sensors.
2.	A check is made to determine whether or not fluid is sensed before the end of the procedure.
3.	If fluid is not sensed before the end of the delivery, either the sensor is faulty or delivery was incomplete.
4.	Failures are reported in the event log.

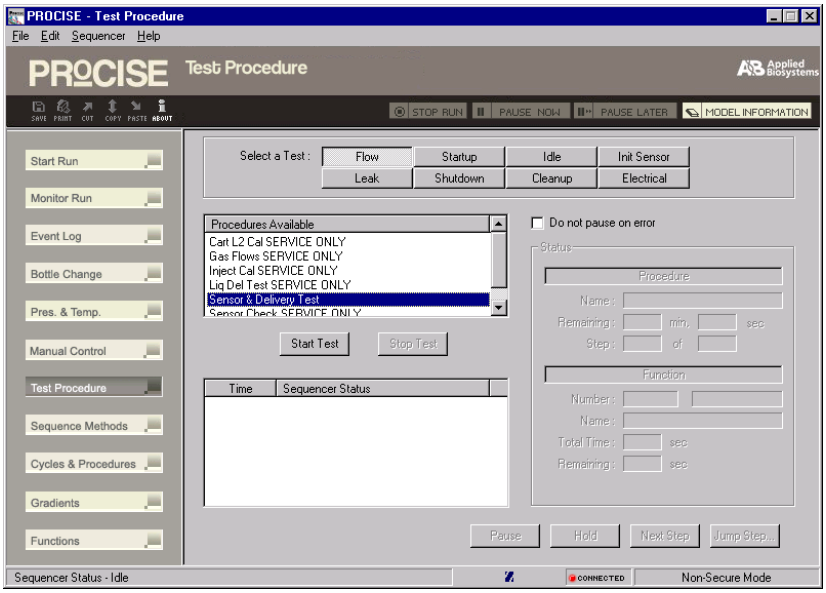
Note: This test should be run while the designated sequencing chemicals are loaded on the instrument. In addition, X2 must contain R5, and X3 must contain methanol.

Running the Sensor and Delivery Test

Note: If the protein sequencer has not run since the last cold start, run the Init Sensor procedure (page 6-7) before continuing with this test.

To run the Sensor and Delivery Test:

1. Select the Test dialog box from the dialog box pop-up menu.



2. Click **Flow**.
3. Select **Sensor & Delivery Test** from the test menu.
4. Select or Deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
5. Click **Start Test**.
6. Allow the test to run to completion.
7. Select the event log dialog box from the dialog box pop-up menu.
8. Review the event log to determine if any delivery errors occurred.

Leak Test Procedures

Leak Test Procedures Overview

The PROCISE control software includes leak tests for:

- Bottles
- Cartridges and cartridge blocks
- The flask and flask blocks
- The waste system
- Regulators

IMPORTANT! Leak tests alter the pressure settings for reagent, solvent, and/or gas deliveries. If a test is interrupted, pressures can remain altered. To reset the default operating pressures, select the Pressures & Temperatures dialog box, and click Default.

Bottle Leak Test Overview

Bottle leak tests are run:

- From the Test dialog box
- From the Bottle Change dialog box

Note: Refer to "Bottle Change Procedure Options" on page 6-8 for a discussion of the Bottle change procedure. this section presents leak testing run from the Test dialog box.

Each bottle leak test performs the following checks:

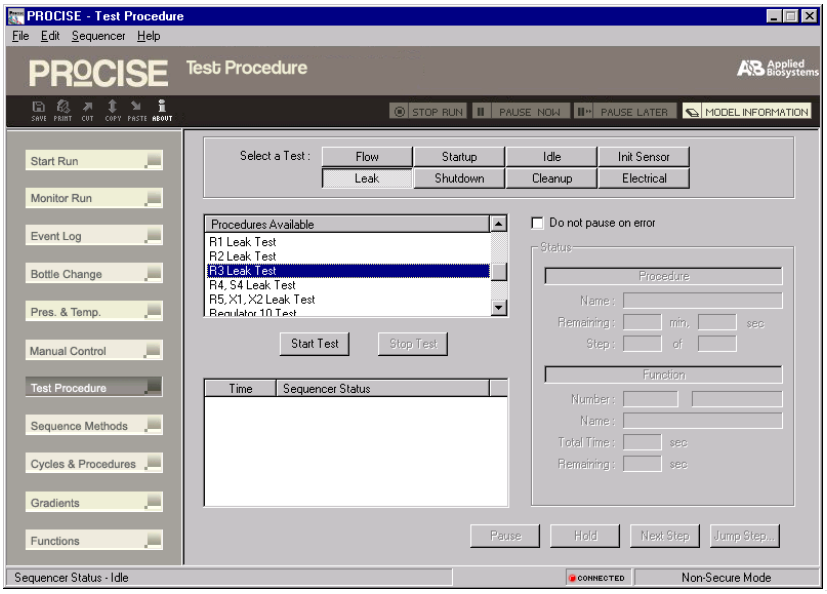
- Pressurization – Checks that the bottle can be adequately pressurized
- Monitor Leak Rate – Measures the pressure drop with the regulator set to zero
- Vent – Checks the venting capability

Test results are reported in the event log at the end of the test. The actual bottle pressure must be within 0.05 psi of the target pressure to pass the leak test.

Using the Test Dialog Box

To run a bottle leak test from the Test dialog box:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Leak**.



3. Select the appropriate bottle from the menu.
4. Click **Start Test**.

Note: Refer to "Creating Tests and Procedures" on page 6-26 for instructions on performing bottle leak tests as part of the bottle change procedure.

Cartridge Leak Test Procedure

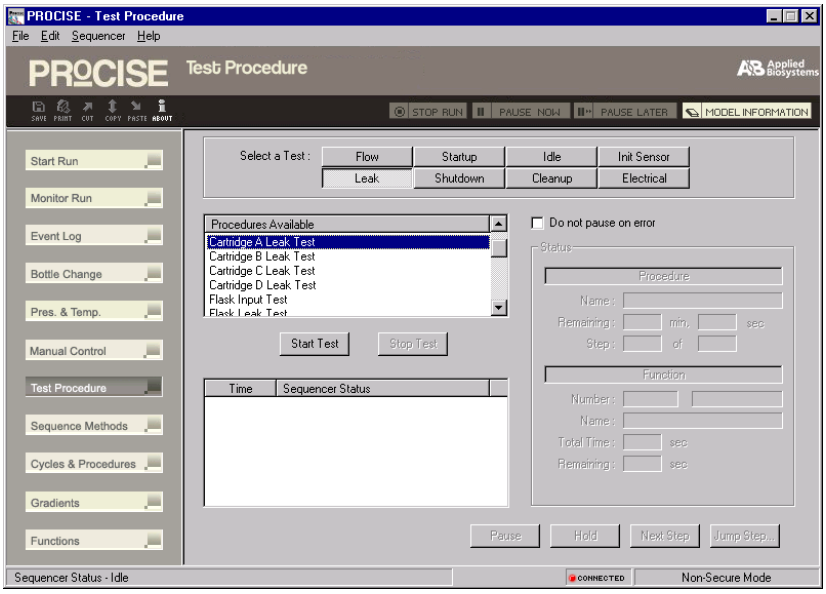
Overview

The Cartridge leak test ensures the leak tightness of each cartridge. The cartridge is pressurized, and the leak rate is monitored.

The actual cartridge pressure must be within 0.07 psi of the target pressure to pass the cartridge leak test. The flow path for a leak test performed on cartridge A is shown in Figure 6-1 on page 6-18.

To run a Cartridge leak test:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Leak**.
3. Select the appropriate cartridge leak test from the test menu.



4. Select or deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
5. Click **Start Test**.
6. Allow the test to run to completion.

   Test results are reported in the event log at the end of the test.

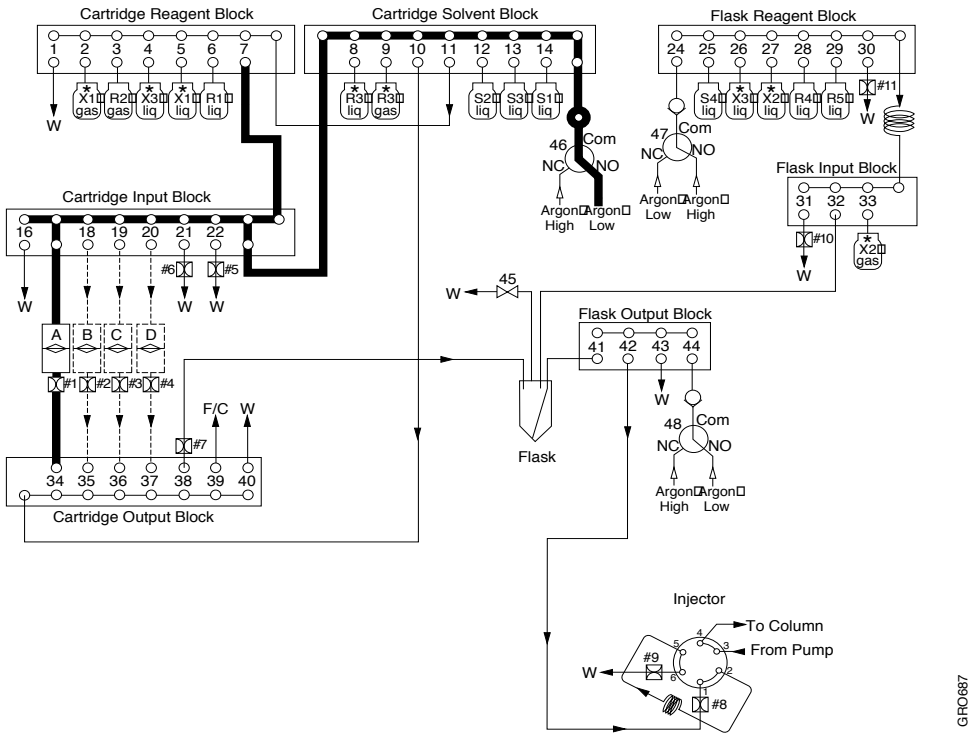


Figure 6-1 Flow path for Cartridge Leak test performed on Cartridge A

Flask Leak Test Procedure

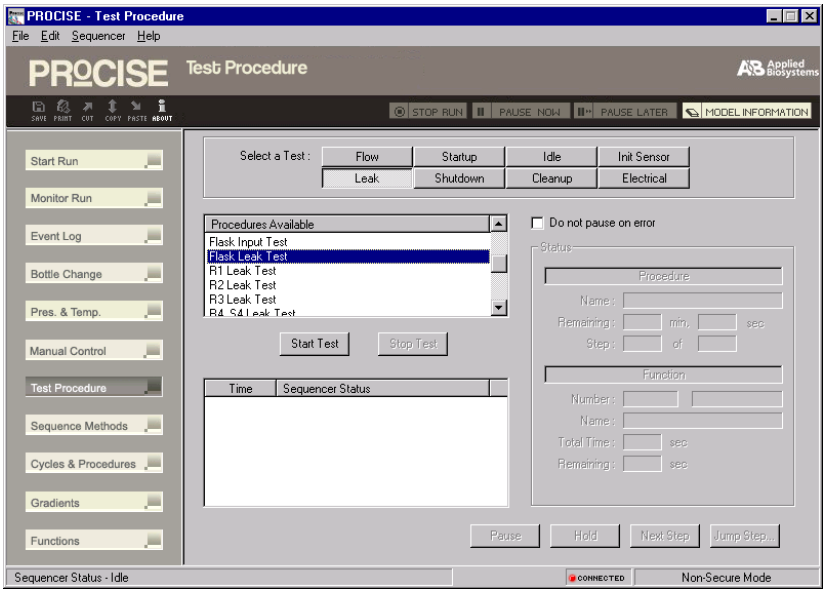
The Flask leak test checks the sealing and venting capability of the flask assembly.

Note: The actual Flask pressure must be within 0.05 psi of the target pressure to pass the flask leak test.

The flow path for this test is illustrated in Figure 6-2 on page 6-19.

To run a Flask leak test:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Leak**.
3. Select **Flask Leak Test** from the test menu.



4. Select or deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
5. Click **Start Test**.
6. Allow the test to run to completion.

   Test results are reported in the event log at the end of the test.

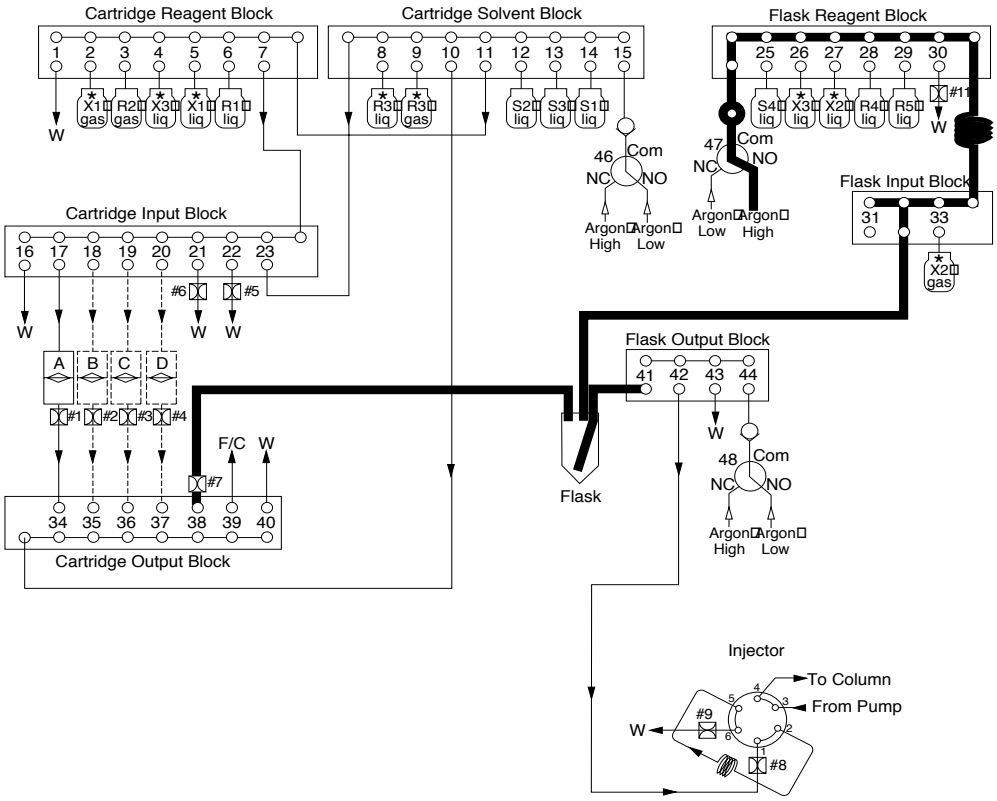


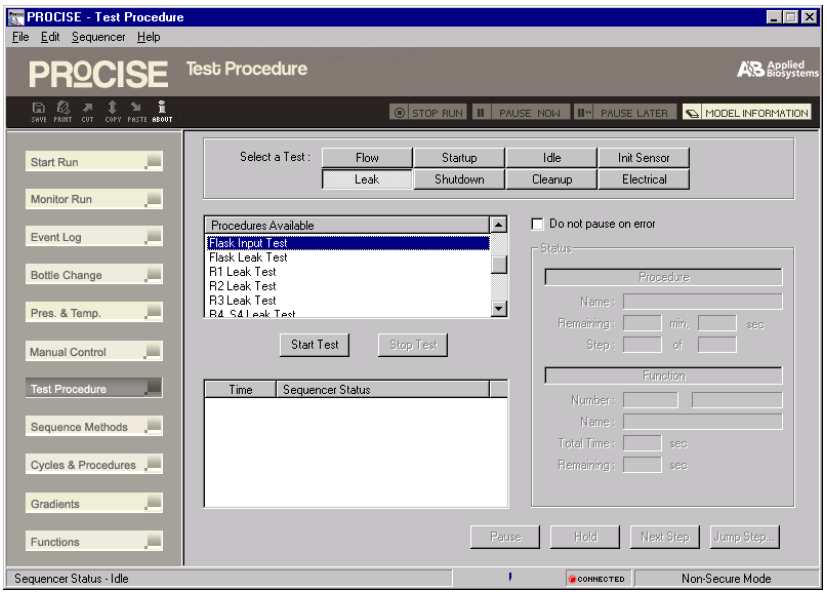
Figure 6-2 Flow path for the Flask Leak test

Flask Input Test Procedure

The Flask Input test is used to leak test both the flask reagent and flask input blocks. The flow path for this test is shown in Figure 6-3 on page 6-21.

To run the Flask Input test:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Leak**.
3. Select **Flask Input Test** from the Test menu.



4. Select or deselect the Don't Pause On Error box. Refer to page 6-2 for information on this option.
5. Click **Start Test**.
6. Allow the test to run to completion.

   Test results are reported in the event log at the end of the test.

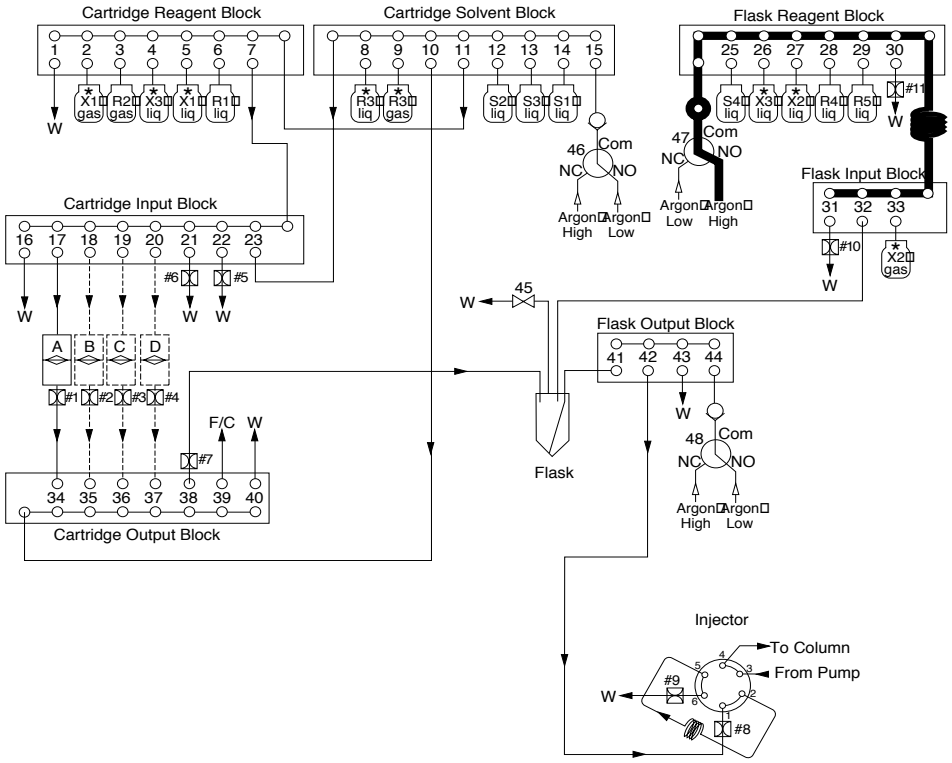


Figure 6-3 Flow path for the Flask Input Leak test

Cartridge Block Leak Tests Procedure

Two Cartridge (valve) Block leak tests are included with the PROCISE control software:

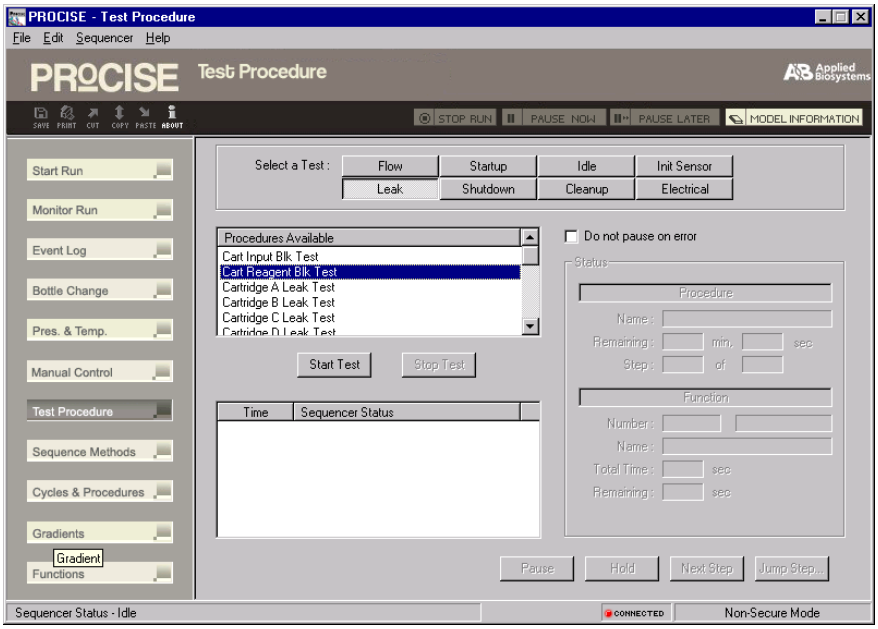
- A cartridge reagent block test
- A cartridge input block test

These tests check the sealing and venting capability of each valve block. The flow paths for these tests are illustrated on page 6-22.

Note: The actual pressure held in the valve block must be within 0.05 psi of the target pressure to pass the valve block leak test.

To run the Cartridge Block Leak test:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Leak**.
3. Select either **Cart Reagent Blk Test** or **Cart Input Blk Test** from the test menu.



4. Select or deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
5. Click **Start Test**.
6. Allow the test to run to completion.

   Test results are reported in the event log at the end of the test.

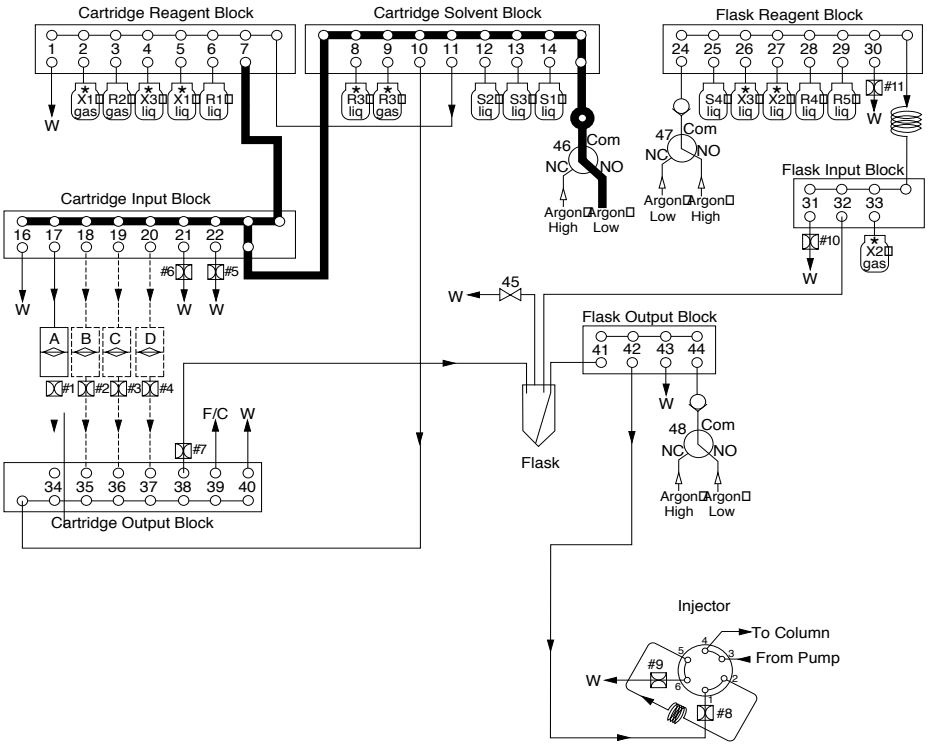


Figure 6-4 Flow path for the Cartridge Input Block Leak test

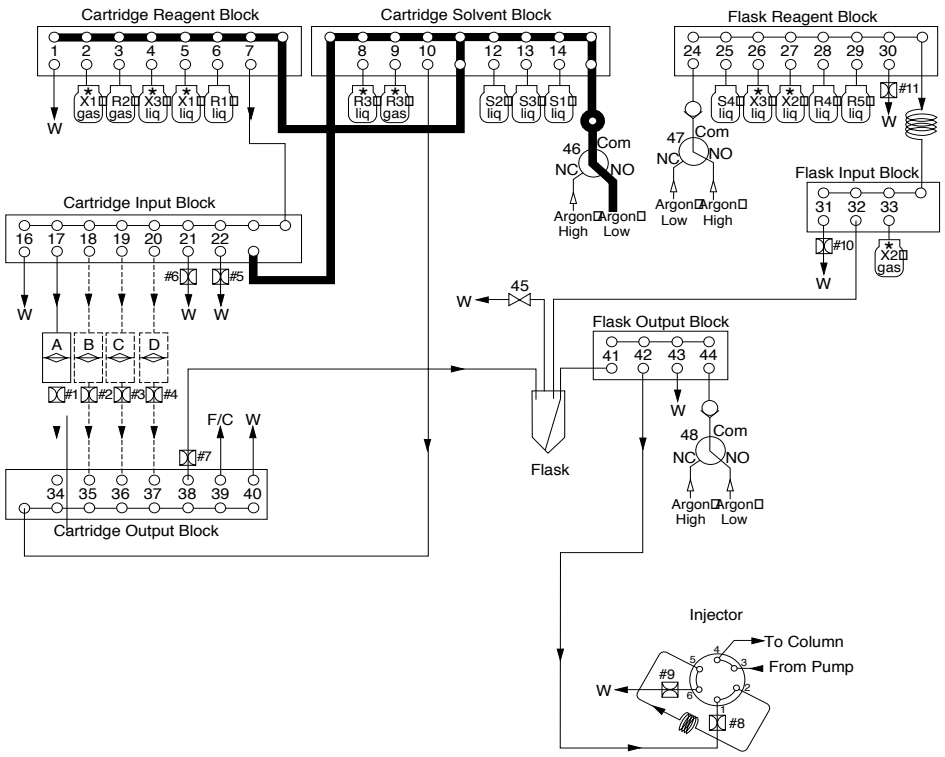


Figure 6-5 Flow path for Cartridge Reagent Block Leak test

Cleanup Procedures

Cleanup Procedures Overview

Five cleanup procedures for the protein sequencer are included in the PROCISE control software:

- Cartridge Line Cleanup
- Clean Transfer Line with X1
- Delivery Line Backflush
- System Cleanout – X3
- System Flush – Argon

Note: The last three procedures listed are used only for a complete system shutdown. A complete system shutdown occurs when all the instruments will be powered down, disconnected and placed in storage. Refer to "Complete System Shutdown Procedure" on page 7-4 in Chapter 7, "Maintenance," for further information on the use of these procedures and a complete system shutdown.

Cartridge Line Cleanup Recommendations:

Run the Cartridge Line Cleanup procedure:

- On a routine basis (weekly or monthly) as part of your regular protein sequencer maintenance
- When chemical noise or background becomes too high and is not due to dirty sample(s). Verify by running a cartridge with no sample.

Cartridge Line Cleanup Overview

This procedure:

- Cleans the reagent, the solvent, the input and output valve blocks with methanol from the S2 position
- Washes the cartridge inlet and outlet lines
- Dries the washed areas

Note: Do not run this procedure when samples are loaded on the protein sequencer.

Cartridge Line Cleanup Procedures

WARNING **CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION **CHEMICAL HAZARD**. **Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To install methanol:

1. Install methanol in the S2 bottle position.
2. Select **Bottle Change** from the dialog box pop-up menu.
3. Select **Bottle 7**, **Chemical S2** from the chemistry menu.
4. Select **Bottle Change for S2** from the Bottle Change Procedure pop-up menu, and click **Change Bottle**.
5. When prompted, remove the S2 bottle and install a bottle of methanol.
6. Click **Continue**.

To clean the cartridge line:

1. Once the bottle change procedure is complete, select **Test** from the dialog box pop-up menu.
2. Click **Cleanup**.
3. Select **Cartridge Line Cleanup** from the list of procedures.
4. Click **Start Test**.
5. When the cleanup procedure is complete, use the Bottle Change procedure for S2 to remove the bottle of methanol, and reinstall the S2 bottle onto the protein sequencer.

Clean Transfer Line with X1 Overview

This procedure removes buildup from the transfer line between the output block of the cartridges to the flask.

Perform this procedure:

- When background becomes excessive
- On a routine basis (weekly or monthly) as part of regular protein sequencer maintenance

Clean Transfer Line with X1 Procedure

IMPORTANT! Install methanol in the X1 bottle position:

Perform this portion of the procedure only if methanol is not already installed in the X1 bottle position.

To clean the transfer line with X1:

1. Select **Bottle Change** from the dialog box pop-up menu.
2. Select **Bottle 10**, **Chemical X1** from the chemistry menu.
3. Select **Bottle Change for X1** from the Bottle Change Procedure pop-up menu, and click **Change Bottle**.
4. When prompted, remove the X1 bottle and install a bottle of methanol if methanol is not already installed in this bottle position.
5. Click **Continue**.

To run the cartridge line Cleanup procedure:

1. Select **Test** from the dialog box pop-up menu.
2. Click the **Cleanup** button.
3. Click **Clean Transfer Line with X1** from the list of procedures.
4. Click **Start Test**.

Electrical Test Procedure

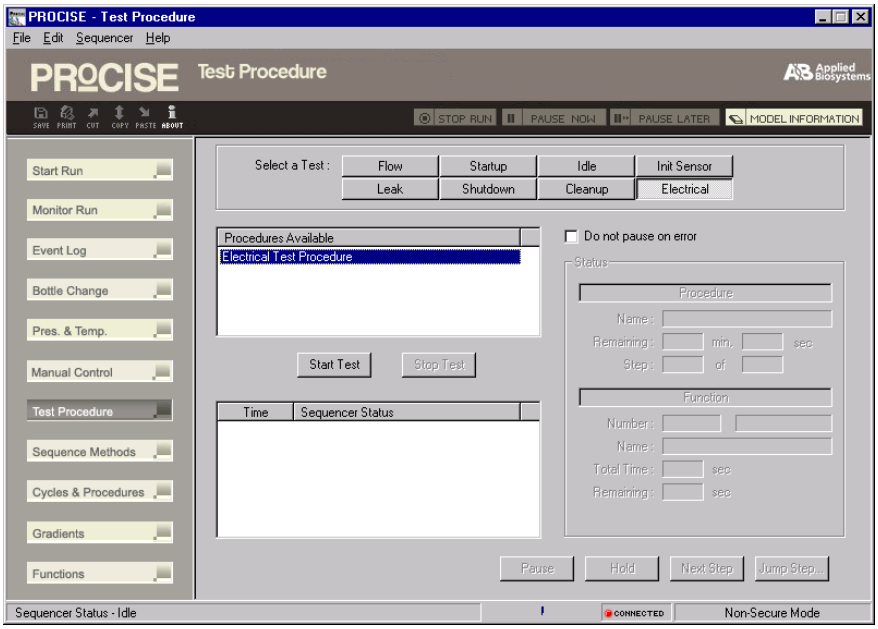
The Electrical test, run automatically every time the protein sequencer is powered up, performs several functions:

- Checks the electrical continuity of key components in the system
- Switches the Rheodyne valve from the load position to the inject position, then back to the load position during step 7 in the procedure
- Reports failures in a dialog box on the screen and in the event log

Electrical Test Procedure

To run the Electrical test:

1. Select the Test dialog box from the dialog box pop-up menu.
2. Click **Electrical**. The Electrical Test Procedure is automatically selected.



3. Select or deselect the **Don't Pause On Error** box. Refer to page 6-2 for information on this option.
4. Click **Start Test**, and allow the test to run to completion.
5. Review the results of the test in the event log.

Creating Tests and Procedures

Overview

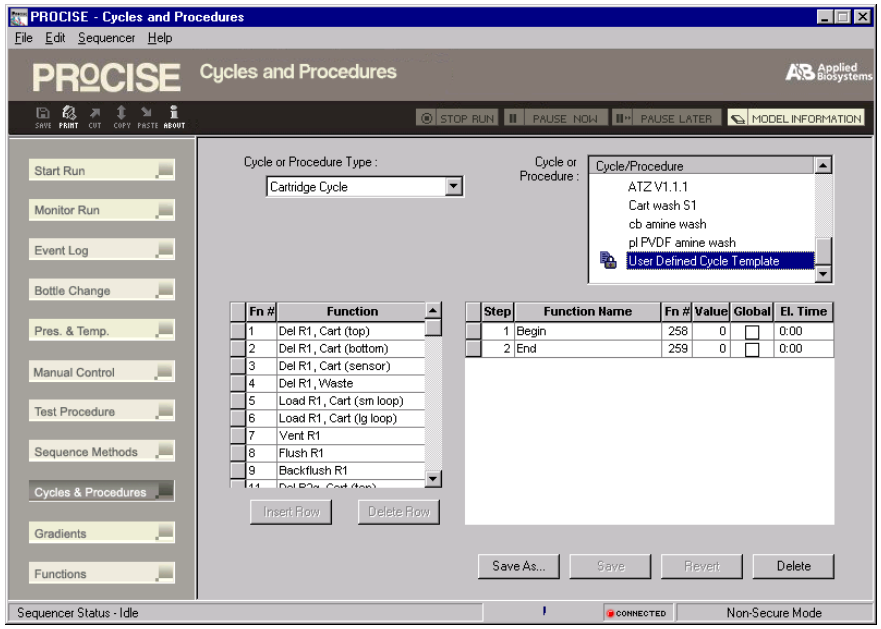
To create your own tests and procedures, you can:

- Save a standard test or procedure under a new, unique file name, then edit the test or procedure.
- Select User Defined Cycle 1 from the Test pop-up menu, then select and insert steps into the test or procedure.

Procedure

To create tests and procedures:

1. Select the Cycles & Procedures dialog box from the dialog box pop-up menu.
2. Select the type of procedure you wish to create from the Cycle and Procedure category pop-up menu.



3. Select the test or procedure you wish to use as a template from the Test and Procedure pop-up menu (page 6-25).
4. Pull down the File menu from the main menu bar, and select **Save Cycle/Procedures As**.
5. Enter a unique name for the new test/procedure, and click **OK**.
6. Edit the steps in the procedure:

If you need to...	Then...
delete a row	1. Highlight the row. 2. Click **Delete Row**.
insert a row	1. Select the function to be inserted from the function list. 2. Highlight the row immediately before the insertion point. 3. Click **Insert Row**.

Follow these guidelines:

- The maximum number of steps allowed per cycle is 100.
- Every cycle must include a Begin step and an End step.
- Deselect the box labeled Global if the global time not is used.
- Enter the function time in seconds in the Value box.

7. Save the procedure:
   a. Pull down the File menu from the main menu bar.
   b. Select **Save Cycle/Procedure**.

Computer Lockup Procedures

Gathering Information During a Lockup

Record the answers to the following questions.

General Questions

- What are the circumstances under which the lockup occurs? This information is critical for determining the cause of the problem.
- What is the sequence of events that preceded the lockup?
- What time did the lockup occur? (morning, overnight, etc.)
- Did any other instrumentation experience a problem?
- If a system lockup occurred in the past, did it occur under the same circumstances? Can the circumstances be reproduced?

Computer Questions

- Does the cursor move?
- Are any screen functions active?
- Is a SequencePro™ Data Analysis Application "Collecting" window open?
- Is the step time counting down on the Monitor Run screen?
- Which steps are displayed?
- What is the most current information in the event log?

Protein Sequencer Questions

- Is the door panel COMM LED lit?
- Is the door panel SEQ LED lit?
- Is the protein sequencer still running? If it is, you will hear the valves clicking.
- Are any of the red Error LEDs on the inner panel lit?
- Are any of the green Status LEDs on the inner panel lit?

Overview – Recovering from a Computer Lockup

These steps will help recover from a computer lockup. Try the following suggestions, one at a time, in sequence until normal operation is restored.

IMPORTANT! If communication is re-established after step 4 and you wish to abort the run, wait 5 minutes before you click Stop. This ensures that all of the data is transferred from the protein sequencer to the computer.

Computer Reset Procedure

To reset the computer:

1. Turn off the computer, wait 5 sec, then turn on the computer.
2. Relaunch the PROCISE application (if it does not automatically launch as part of the startup routine).
3. Turn off and turn on the protein sequencer.
4. Repeat step 1 (reset the computer).

Reset the Protein Sequencer

IMPORTANT! Resetting (cold booting) the sequencer as described below is a last resort because it erases the sequencer memory including the current run conditions, chromatogram data and the Event buffer. The Event buffer may contain valuable information that has not yet been transferred to the computer Event Log file on the hard disk.

To reset the sequencer:

1. Turn off the sequencer.
2. Unplug the MEL card (lefthand side, upper rear corner).
3. Turn on the sequencer.
4. Turn off the sequencer.
5. Plug in the MEL card.
6. Turn on the sequencer.
7. Reboot the computer.

IMPORTANT! The message "Execution of Cold start (all RAM has been initialized)" should appear in the event log. If it does not, make sure that jumper, W6, has been removed from the CPU printed circuit board.

Gathering Information After a Lockup

Overview

After a lockup, gather answers to the questions below.

Important Information

What version of software or firmware is installed for the following?

- The MEL card
- The PROCISE operating software
- The SequencePro software

Do you have printouts of the relevant sections of the event log?

SequencePro Event Log

Follow the steps below to print out the SequencePro event log complete with service information.

To print the SequencePro event log:

1. While in the SequencePro application, go under View and select **Event Log**.
2. Select **Print** from the File menu.

Recovering from a Power Failure

Overview

If a power failure occurs while the sequencer is running:

- An error is generated in the Event buffer.
- The run pauses at the end of the cycle once the power returns.

If you wish to continue the run, follow the procedure below.

After a Power Failure

To recover from a power failure:

1. Check that all the instruments are powered up and that the PROCISE application has automatically launched.
2. Scroll to the Pressures & Temperatures dialog box, and click **Execute** to turn the heaters back on. The respective heater LEDs on the front panel should illuminate.
3. Scroll to the Start Run dialog box, and click **Resume**.
4. Relaunch the SequencePro application.
5. The message “File already exists” may be generated now or later during the run.

   If this occurs:

   a. You will be prompted to rename the SequencePro data file.

   b. Rename the file at this time.The new file you create will contain all the data from the original file.
6. Delete the original SequencePro file.

Fluid Sensor Data Files

- A separate data file can be generated for each fluid sensor.
- The information in these files is similar to that in the event log; however, it is reported every time the sensor is used, not just when an error occurs.
- The information in these files can be used to help determine the cause of intermittent delivery problems.
- Sensor data files are stored in the PROCISE folder. The PROCISE folder is located in the Preferences folder in the System folder.

Note: Fluid sensor data files can grow to 1 Mb each (11 Mb total) if the "Always report sensor data" and "Report valve status" options are selected (turned on) all the time. Therefore, use this feature only if you suspect a delivery problem. Delete the files once the problem is resolved.

Generating Fluid Sensor Data Files

To generate fluid sensor data select the Always report sensor data box in the Preferences window. When selected, an X appears in the box.

Stop Generating Fluid Sensor Data

To stop the generation of fluid sensor data:

1. Deselect the "Always report sensor" data box in the Preferences window.
2. Delete the fluid sensor data files once the delivery problem is resolved.

Note: Quitting the PROCISE application automatically deselects the "Always report sensor data" box.

Opening Fluid Sensor Data File in Microsoft Excel

Although Microsoft® Excel version 5.0 is not preloaded onto the system's computer, it is the most suitable application for opening fluid sensor data files.

To open fluid sensor data files in Microsoft Excel:

1. Launch the Excel application.
2. Select **Open** from the File menu.
3. Highlight the desired sensor data file, and select **Open**.

Format the Sensor Data File

After opening the file, follow the steps below.

To format the Data Sensor file in Excel:

1. Choose the desired formatting.
2. Fit all the horizontal information on the screen.
   a. Highlight the complete document by clicking the box at the upper-left corner in the window.
   b. From the Format menu, select **Columns**.
   c. Then select **Autofit Columns** from the pop-up menu. Display the column title bar while scrolling vertically.
   d. Move the cursor over the black box just below the right corner of the window bar. The arrow becomes two parallel lines.

e. Click and hold the mouse button while moving the cursor downward until it is immediately below the column title row.

f. Release the mouse button to split the screen.

Opening Fluid Sensor Data Files Using a Text Editor

As long as the sensor data files are small, they can be opened with NOTEPAD, which is installed on the computer as part of the Windows® 2000 operating system.

To open a Fluid sensor data file with NotePad:

1. From the Windows 2000 Start menu, scroll to Programs and select Accessories.
2. Double-click the NotePad icon to launch the application.
3. Select Open from the File menu.
4. Highlight the desired sensor data file, and select Open.

Note: If the file is too large for NOTEPAD to open, use an alternative text editor, such as Microsoft Word or WORDPAD.

The format in which NOTEPAD displays the sensor information is not ideal. You can adjust the size and the font of the title bar. Reducing the type size and/or orientation allows each step of information to be reported on the same line.

Maintenance 7

This chapter describes instrument maintenance procedures for idle time, startup, and shutdown periods. Hands-on maintenance and troubleshooting procedures for the protein sequencer, the column, 140 pump and computer are also included.

The following topics are contained in this chapter:

Startup and Shutdown, Replacing Argon Cylinders, and Testing HPLC

Procedures After Idle Time

Certain procedures should be executed if the protein sequencer instrument is to be idle for any length of time, even one day.

The procedures you follow are based on the length of time the system will be idle.

Table 7-1 Idle time recommendations

Length of Idle Time	Recommended Procedures
1 day	• Before leaving the system idle, no special treatment is required prior to leaving the system idle. • Before using the system again: – Run the System Cleanout –X1-X2 Procedure. – Run the Cartridge Line Cleanup Procedure. This procedures washes the valve block, sensor lines, loop, and injector. Refer to “Cartridge Line Cleanup Procedures” on page 6-24, for instructions on performing this procedure.
1 to 7 days	• Before leaving the system idle. no special treatment is required. • Before using the system again: – Run the Startup Procedure. – Purge the 140 pump. – Run the Cartridge Line Cleanup procedure. – Run one sequencing cycle on each cartridge before loading samples. **Note:** Refer to Chapter 6, Tests and Procedures for instructions on performing these procedures.
8 to 14 days	• Before leaving the protein sequencer idle: – Configure the Idle Procedure to run every 8 hours while the protein sequencer is idle. The argon supply must remain connected to the protein sequencer to run this procedure. – Choose one of the following before leaving the HPLC components of the system idle: – Set up the pump to free run in manual mode at 5 to 10 µL/min, 50 %B, or – Clean and shut down the 140 pump and UV detector. Refer to Chapter 6, Tests and Procedures and the 140 pump user’s manual for more information on these procedures. • Before using the system again, follow the setup procedures in Chapter 2, “Preparing the System.” **Note:** Be sure to load fresh chemicals onto the protein sequencer and prepare fresh solvent for the 140 pump.

Table 7-1 Idle time recommendations *(continued)*

Length of Idle Time	Recommended Procedures
More than 14 days	• Before leaving the system idle: – Run the Short-term Shutdown procedure. – Empty and rinse the waste bottle on the protein sequencer. – Set up the 140 pump to free run in manual mode at 5 to 10 µL/min, 50 %B. **Note:** Refer to Chapter 6, Tests and Procedures and the 140 pump user's manual for more information on these procedures. • To start the system up again: – Follow the setup procedures in Chapter 2, "Preparing the System." **Note:** Be sure to load fresh chemicals onto the protein sequencer and prepare fresh solvent for the 140 pump.
Complete system shutdown	• To completely shut the system down: – Follow the procedure "Complete System Shutdown Procedure" on page 7-4. • To start the system up again: – Follow the setup procedures in Chapter 2, "Preparing the System."

Complete System Shutdown Procedure

A complete system shutdown means that the instruments will be disconnected from the argon and electrical support.

Applied Biosystems recommends that you perform the following procedures for a complete system shutdown:

1. Purge the 140 pump dry.
2. Run the Delivery Line Backflush procedure.
3. Run the System Cleanout X3 procedure.
4. Run the System Flush–Argon procedure.
5. Remove all reagent and solvent bottles from the protein sequencer.

CAUTION If the reagent and solvent bottles are not removed before a complete system shutdown, the protein sequencer valve blocks may sustain damage.

Purging the 140 Pump Dry

WARNING **CHEMICAL HAZARD. A3 (3.5% tetrahydrofuran in water)** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation, central nervous system depression, and liver and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD. B2 (12% isopropanol and acetonitrile)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD. B3 (18% tetrahydrofuran in acetonitrile)** is a flammable liquid and vapor. It may be harmful if swallowed. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To purge the 140 pump dry:

1. Replace the 140 pump solvent A3 with HPLC grade or deionized water.
2. Purge both pumps three times at 100%.
3. Run the 140 pump in manual mode at 50 µL/min, 50% B for at least 30 min.
4. Place both solvent lines into a bottle of solvent B2. Purge both pumps three times at 100%.
5. Disconnect the line at the injector, and place the end of the line in the waste bottle.
6. Free run the 140 pump at 200 µL/min, 50% B for 5 min.

7. Place both solvent lines into clean, dry bottles.
8. Purge both pumps three times at 100%.

Backflushing the Delivery Line

The Delivery Line Backflush procedure:

- Prepares the system for removal of all reagent bottles
- Backflushes all reagents and solvents from the delivery lines

When the procedure is finished, remove and empty all reagent and solvent bottles.

To run the Delivery Line Backflush procedure:

1. From the Test dialog box, click **Cleanup**.
2. Select the Delivery Line Backflush procedure, and click **Start Test**.
3. When the test is finished, remove all solvents and reagents from the protein sequencer.
4. Install a bottle of HPLC grade methanol in the X3 bottle position.
5. Install empty bottles in all the other bottle positions.

Running System Cleanout X3

The System Cleanout X3 procedure:

- Cleans the protein sequencer
- Requires that the heptane from the X3 bottle position be replaced with a bottle of 100% methanol
- Empty bottles **must** be placed in all the other bottle positions
- Washes all valve blocks, delivery lines, reaction cartridges, loops, injectors, and reagent bottles with methanol

To run the System Cleanout X3 procedure:

1. Select the System Cleanout X3 procedure, and click **Start Test**.
2. When the procedure is finished, replace the bottle of methanol with an empty bottle.

Running System Flush with Argon

The System Flush Argon procedure:

- Flushes and dries all protein sequencer flow paths with argon
- Resets pressure regulators to the default pressure settings

To run the System Flush–Argon procedure:

1. Select the System Flush–Argon procedure, and click **Start Test**.
2. When the procedure finishes, remove the waste bottle from the protein sequencer.
3. Empty, rinse, and reinstall the waste bottle.
4. Turn all the instruments off and disconnect them from the power supply.

Startup Procedure After a Complete System Shutdown

Recommendation

We recommend that you perform the following procedure to start the system after a complete shutdown.

Note: For more information on these procedures, refer to Chapter 6, "Tests and Procedures," and the *ABI 140D Microgradient Delivery System User's Manual* (P/N 903586).

Starting the System

To start the system:

1. Connect the instruments to the power supply, and turn them all on.
2. Load fresh reagents and solvents onto the protein sequencer using the Startup procedure.
3. Prepare and load fresh solvents onto the 140 pump.
4. Purge the pump.
5. Run a blank gradient (Run Gradient cLC) from the Start Run dialog box.
6. Check the HPLC components of the system for leaks while the blank gradient is running.

Replacing the Argon Cylinder

Replace the argon cylinder when the tank pressure falls below 100 psi.

CAUTION **CHEMICAL HAZARD. Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Replacing the argon cylinder consists of three steps:

- Removing the old cylinder
- Installing the new cylinder
- Leak testing the new cylinder

Items Required to Replace the Argon Cylinder

The following items are required to replace the argon cylinder:

- Large wrench for removing argon regulator
- Argon cylinder valve key (if necessary)
- Cylinder blanking plug removal tool (if necessary)
- Teflon™ tape (if necessary)
- Safety goggles

Removing the Old Cylinder

WARNING **EXPLOSION DANGER.** Gas cylinders must be securely fastened upright to a wall, cylinder truck, or cylinder post. Always replace the cylinder cap when the cylinder is not in use, and when it is being moved. Never place cylinders in hallways where they could be hit by a forklift or struck by a falling object. Do not allow grease, oils, or other combustible materials to touch any part of a cylinder.

To remove the old argon cylinder:

1. Note the current operating pressure on the regulator.
2. Turn off the argon tank shutoff valve.
3. Open the purge valve on the rear of the instrument to bleed off any residual pressure.
4. Remove the regulator, and carefully set it down.
5. Remove the old cylinder.:

Installing the New Cylinder

To install the new argon cylinder:

1. Fasten the new cylinder securely in place.
2. Wearing safety goggles, briefly open and close the cylinder shutoff valve to remove any debris that may have settled.
3. Screw the regulator onto the new cylinder. Use Teflon tape if appropriate.
4. Open the cylinder shutoff valve. Gas will start to flow out of the instrument purge valve.
5. Close the tank regulator by turning the adjustment knob fully counterclockwise.

Testing the New Cylinder for Leaks

To leak test the new argon cylinder:

1. Close the tank shutoff valve, and wait 30 sec.
2. Using tape, mark where the needle is registering on the tank high-pressure regulator gauge.
3. Wait 1 min, and note the reading on the high-pressure gauge.
4. If the pressure has visibly dropped, there is a leak.

   Determine the cause of the leak, and repair it appropriately.

   If there are no leaks, turn the tank shutoff valve back on.
5. Adjust the tank regulator to the recommended operating pressure of 65 psi.

Testing the HPLC Components of the System

You can use the Run Gradient cLC sequencing method to:

- Test the integrity of the pumping system
- Test the solvent-mixing efficiency

This method automatically:

- Starts the 140 pump
- Equlibrates the column at initial conditions
- Starts the gradient

Note: No injection takes place.

Running the Gradient cLC Sequencing Method

To use the Run Gradient cLC sequencing method:

1. Purge the 140 pump one time at 100%.
2. In the Start Run dialog box, set up a run.
   a. Set the Cartridge A Run Order to **1**.
   b. Enter a unique file name for the run.
   c. Select **Run Gradient cLC** as the method.
   d. Set the Number of Cycles to at least 1.
3. Click **Start Run**.

Interpret the results when the run is finished:

If the gradient profile...	Then...
is similar to the one shown in Figure 7-1 on page 7-10.	the HPLC components of the system are functioning properly.
differs significantly from Figure 7-1	troubleshoot the HPLC components of the system to find the source of the problem.

Gradient Profile with No Injection

Figure 7-1 shows a gradient profile in which the system's HPLC components are running normally.

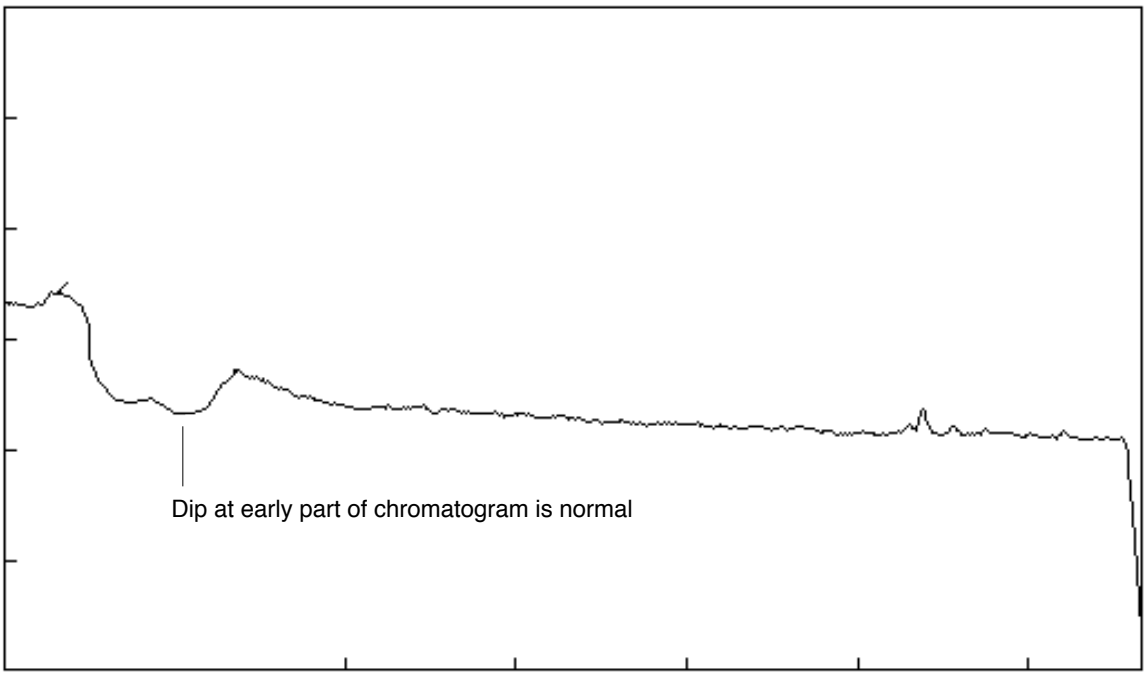


Figure 7-1 Gradient profile with no injection

Protein Sequencer Maintenance

Internal Components of the Protein Sequencer

Accessing Components

WARNING **ELECTRICAL SHOCK HAZARD.** Severe electrical shock can result from removal of the rear panel. Therefore, do not remove the rear panel of the protein sequencer. The panel should be removed by an Applied Biosystems service engineer only.

To access the internal components of the Procise cLC Protein Sequencer:

1. Raise the bezel.
2. Remove the top panel.
3. Remove the side panels.
4. Lower the plumbing plate.

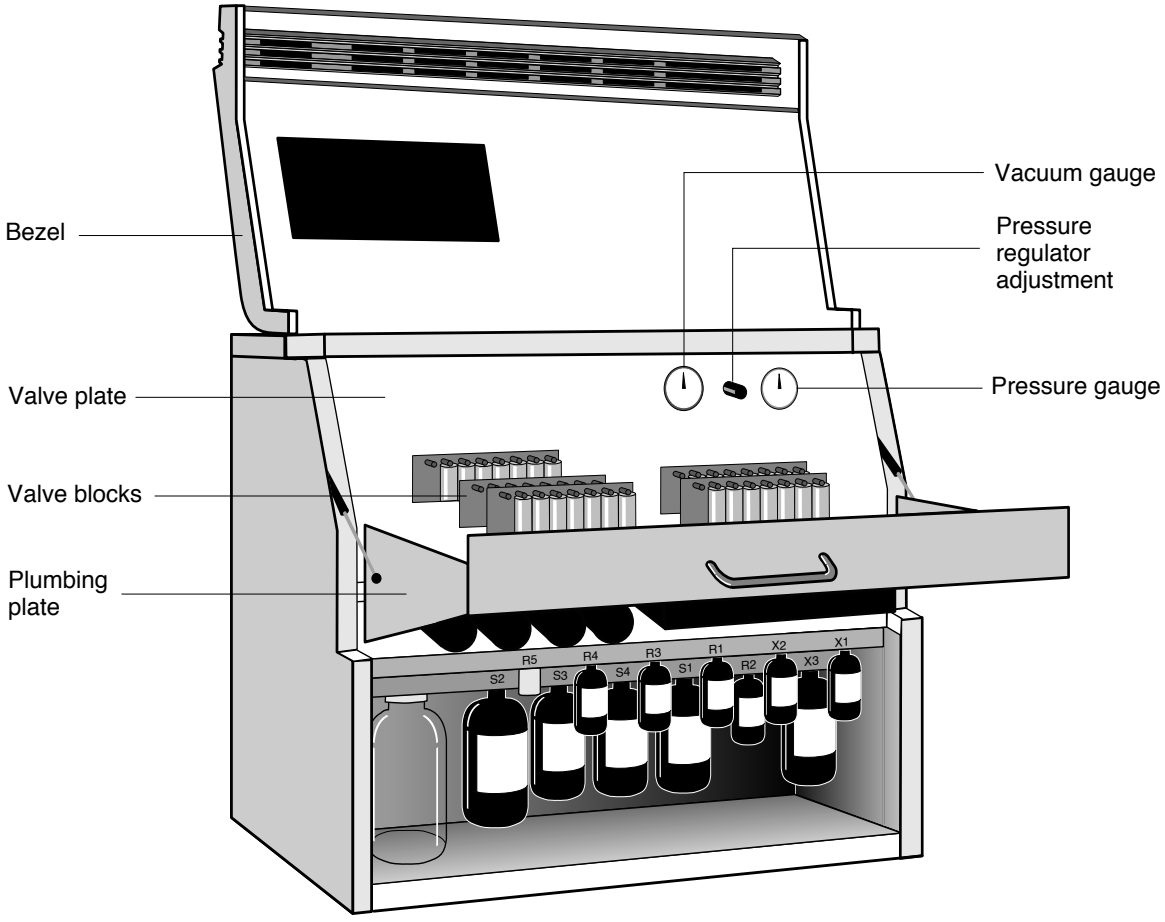


Figure 7-2 User accessibility to the internal components of the protein sequencer

Miscellaneous Maintenance Recommendations

Maintaining the Conversion Flask Vial

Over time, the conversion flask vial and pickup tube will need to be replaced.

- Replace the pickup tube every time the conversion flask vial is replaced. Refer to "Replacing the Conversion Flask Vial" on page 7-15 for instructions.
- A dirty conversion flask and pickup line can negatively impact the transfer of sample from the flask to the sample injection loop.
- Spare parts are in the flask maintenance kit (P/N 401990). The vial and pickup tube in this kit are matched, eliminating the need for pickup tube adjustment.

Instructions for removing the conversion flask are included in "Replacing the Conversion Flask Vial" on page 7-15.

Cleaning the Reaction Cartridge

Clean the glass reaction cartridge blocks by pyrolysis if they become contaminated. This procedure requires:

- One shallow borosilicate glass dish
- Furnace or oven that can achieve a temperature of 630 °C
- New, clean, disposable powder-free latex gloves when handling the glass blocks

To pyrolize the glass cartridge blocks:

1. Remove the glass cartridge blocks from the instrument and place them into a borosilicate glass dish. Make sure that the sealing surfaces of each block are facing upward.
2. Place the borosilicate glass dish into the furnace and set the temperature to 630 °C. maintain the temperature setting at 630 °C for at least two hours.
3. Turn off the furnace when cleaning is finished.

   Hot. Allow the furnace to cool down, preferable overnight, before removing the blocks and reinstalling them on the instrument.l

Cartridge Valve Block Maintenance

- Cartridge valve blocks can be rinsed with acetone to help reduce non-amino-acid background.
- Follow the acetone rinse with a methanol rinse.

See Chapter 6, Tests and Procedures for instructions.

CHEMICAL HAZARD. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CHEMICAL HAZARD. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Transfer Line Cleaning

The Transfer Line Cleanup procedure cleans the lines between the output block of the cartridges to the flask. Perform this procedure:

- When background becomes excessive.
- On a routine basis (weekly or monthly) as part of regular protein sequencer maintenance.

This procedure is listed in Chapter 6, Tests and Procedures.

Injection System Maintenance

Replace the injector rotor seal once a year. This procedure is listed on page 7-18.

Cartridge Valve Blocks/Line Maintenance

Use the Cartridge Line Cleanup procedure to help reduce non-amino-acid background by cleaning the valve blocks and lines.

Refer to Chapter 6, Tests and Procedures for information on this procedure.

Purpose of Cleaning

The Cartridge Line Cleanup procedure:

- Cleans the reagent, solvent, input, and output valve blocks from the S2 position with methanol
- Thoroughly washes the cartridge inlet and outlet lines
- Thoroughly dries the washed areas

When to Perform

Run the Cartridge Line Cleanup procedure:

- As part of your routine, regular protein sequencer maintenance (once a month, for example).
- When chemical noise or background becomes too high to verify that the noise is not due to a dirty sample or samples, run a cartridge with no sample.

Installing New Firmware (Replacing the MEL Card)

Overview

The firmware for the protein sequencer resides on the MEL card.

Note: During the lifetime of the protein sequencer, Applied Biosystems may release new versions of firmware.

Items Required

You need MEL card version 1.01 (P/N 604256).

IMPORTANT! If a different version of the MEL card is installed, the RAM is erased when the instrument is powered up, resulting in the loss of the fluid sensor dry readings.

Procedure

To replace the MEL card:

1. Turn the main power switch off.
2. The MEL card protrudes from the upper, rear, left hand side of the protein sequencer chassis. Press the button adjacent to the MEL card to eject it.

   Note: The label on the MEL card may or may not face front.

   a. Gently push the new MEL card into place.

   b. If it does not seat, turn it over and try again.
3. Once the MEL card is properly seated, turn the main power switch on.
4. If the card is functioning correctly, the LED labeled Ready under the front door visor will light in approximately 15 sec.

Replacing the Conversion Flask Vial

When to Replace

Replace the conversion flask vial whenever white residue is visible on the surface of the glass and in the pickup tube.

WARNING **CHEMICAL HAZARD**. Wear appropriate protective eyewear, clothing, and gloves.

Items Required

The following items are required to replace the conversion flask vial:

- Flask Maintenance Kit (P/N 401990)
- Tweezers
- 6–12 in. length of PEEK tubing

Replacing the Conversion Flask Vial

CAUTION Do not scratch the Kel-F sealing surface.

To remove the flask:

1. Remove the flask vial from the protein sequencer.
2. From the Pressures & Temperatures dialog box, turn off the flask heater, and allow the flask assembly to cool until it is comfortable to touch.
3. Remove the pickup tube, and discard it appropriately. Do not reuse the old pickup tube.
4. Unscrew the knurled knob underneath the flask vial. A constant-force spring, the vial, and a Teflon seal should drop out of the housing.

If...	Then...
the vial does not slide out	thread a piece of PEEK tubing through the pickup line hole and push it out.
the seal remains inside the housing	grip the lip of the seal with tweezers, and pull to remove it.
the vial is stuck	place a service call.

Installing a New Flask Vial

To install a new flask vial:

1. Place a new Teflon seal, lip downward, on top of the vial.
2. Insert the vial into the housing.
3. Install the new pickup tube. The gap between the tip of the tube and the bottom of the vial should be approximately 1 mm.
4. Run the Flask Leak test from the Test dialog box.
5. Optimize the pre- and postconversion dry-downs by running the Flask Optimization cLC method.
6. From the Sequence Methods dialog box, run the PTH-Standards cLC method three times to condition the vial.

Replacing the Ratchet Cap Assembly Receptacle

Items Required

To replace the Ratchet Cap Assembly Receptacle, you need the following items:

- 1/4-in. socket or open-end wrench
- Ratchet cap

The part numbers for the Ratchet cap are shown in Table 7-2.

Table 7-2 Part numbers for the Ratchet Cap

Ratchet Cap Type	Part Number
2 oz.	3557
8 oz.	3558
16 oz.	3559

Replacing the Ratchet Cap Assembly Receptacle

Follow the procedure below to replace the Ratchet Cap Assembly Receptacle. Refer to Figure 7-3 on page 7-17 as you perform this procedure.

To remove the Ratchet Cap Assembly Receptacle:

1. Backflush the delivery line(s) into the reagent bottle, using the appropriate backflush function(s).
2. Turn off the protein sequencer.
3. Remove the appropriate bottle(s).
4. Lower the plumbing plate to expose the valve blocks.
5. Remove the two 1/4-in. standoff nuts and washers from the housing.
6. Lift the housing over the two studs.
7. Loosen the ratchet cap lid, and remove the ratchet cap receptacle.

To install the new ratchet cap:

1. Screw the lid (P/N 3560) into the new ratchet cap receptacle.
2. Place the assembly (ratchet cap lid, insert, and gasket) into the housing.
3. Place the wave spring (P/N 2571) on the underside of the ratchet cap assembly.
4. Reinstall the two washers and 1/4-in. standoff nuts that hold the ratchet housing in place.
5. Reinstall the bottle(s).
6. Power up the protein sequencer.

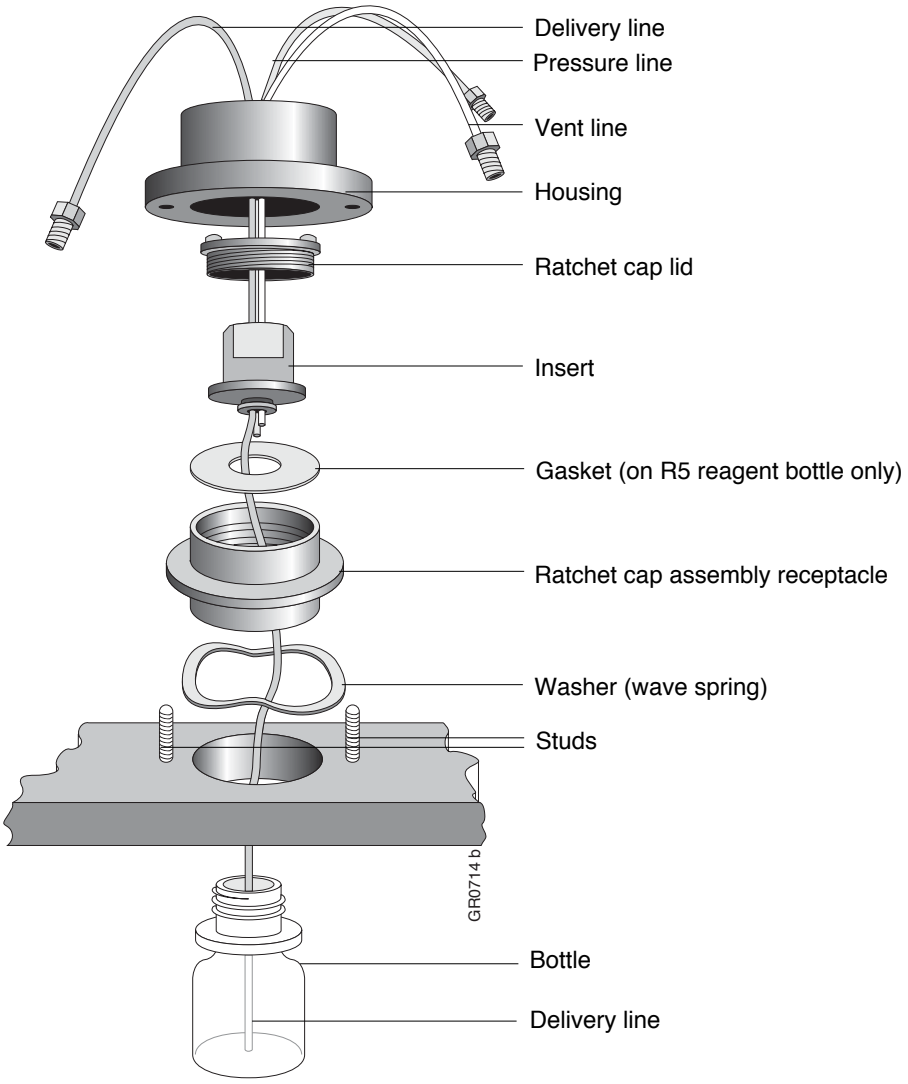


Figure 7-3 R5 reagent bottle ratchet cap assembly

Replacing the Injector Rotor Seal

Items Required

To replace an injector rotor seal, you need the following items:

- 9/64-in. hex wrench
- 1/4-in. wrench
- Rotor seal (P/N 173-0015)
- Isolation seal (P/N 173-0014)

Note: We recommend changing the injector rotor seal once a year.

Removing the Old Injector Rotor Seal

To remove the old injector rotor seal:

1. Remove the plumbing plate from the protein sequencer.
2. Loosen the pretee fittings holding the Injector Full and Load sensors in place.
3. Slide the sensors up the tube and out of the way.

   IMPORTANT! Do not disconnect the lines from the stator unless you need more room.

4. Loosen the three hex screws, and pull the stator from the assembly.

   The two hex screws holding the retainer ring in place are now exposed. Loosen and remove the two screws.

   Note: The stator face will either come off with the stator or remain with the valve assembly. The three dowels in the stator face orient it correctly on the stator.

5. Pull off the retainer ring by screwing two of the hex screws a little way into two of the threaded bores on the retainer ring.
6. Grasp the screws, and pull the ring away from the rest of the assembly.
7. Pull the rotor seal off the four location pins with your finger and thumb.

Installing the New Injector Rotor Seal

To install the new injector seal:

1. Start the procedure.
   a. Inspect the white isolation seal behind the rotor seal.
   b. Replace the seal if it is worn.
2. Inspect the rear of the original rotor seal.
   a. Note the location of the impression made by the shaft bore.
   b. Orient the new rotor seal the same way.
3. Reassemble the injector.
4. Inspect the stator face for scratches. Replace it if necessary.
5. Reposition the sensors.
6. Tighten the pretee fittings that hold the sensors in place.
7. Reinstall the plumbing plate.

Testing the Conversion Flask Assembly for Leaks

The following procedure is another method for leak testing the conversion flask. This method is more stringent than the Flask Leak test run from the Test dialog box.

Leak Testing the Conversion Flask

To leak test the conversion flask:

1. From the Manual Control dialog box, activate Flask Function 171, Del S4, Flask, until the liquid just starts to drip into the flask vial.
2. Activate Function 213, Dry Flask, for 5 sec.
3. Select **None** for the function fields, and activate valves 41, 44 and 48.
4. Examine the flask. After some initial bubbling, all bubbling should cease in 1 min.
   If the bubbling continues, a leak is present.
5. Activate Function 215, Empty Flask, for 10 sec.

Testing the Injector for Blockages

Checking the HPLC Flow Paths

To check the HPLC flow paths for blockages:

1. Remove the inlet line from the column and place it in a beaker.
2. On the 140 pump control panel, press the Manual key to enter Manual mode, and free run the instrument.
3. Set the flow rate to 50 µL/min and the %B to 50.
4. Monitor the pressure for 5 min. The pressure should not rise above 100 psi.
5. If the flow path remains blocked, determine the source of the blockage by breaking fittings in the flow path consecutively from the injector to the dynamic mixer inside the 140 pump.
6. From the Manual Control dialog box on the computer, toggle the injector position by activating Function 223. The pressure should not vary by more than 5 psi. If it does, the sample injection loop may be blocked.
7. Stop the 140 pump, and reinstall the column inlet line.

Testing Three-Way Valves

Overview

If the three-way valve fails to switch from the low-pressure input to the high-pressure (5.5 psi) input during a flush function, the effectiveness of the flush will be compromised.

Similarly, if the valve allows high pressure to bleed into the common path, an overdelivery can occur.

Items Required

You need a 5/16-in. wrench for the three-way valve test procedure.

Testing Three-Way Valves

To test a three-way valve:

1. From the Pressures & Temperatures dialog box, set the low-pressure input (the Set pressure) to the respective three-way valve to 0.
2. From the Manual Control dialog box, turn on the three-way valve by entering the valve number (46, 47, or 48) in the Additional Valves field.
3. Set the Cartridge and Flask function numbers to 0 (None).
4. Click **Execute**.
5. Remove the three-way valve output line from the valve block, and place the end of the line in a beaker of water.
   a. If the valve has switched correctly, a fast stream of bubbles will flow from the tube.
   b. From Manual Control, turn the three-way valve off by clicking **All Off**. The flow of bubbles should stop.
   c. From the Pressures & Temperatures dialog box, reset the input pressure to the correct value. A slower stream of bubbles should flow from the line.

   IMPORTANT! If any of these tests fail, replace the three-way valve.
6. Reconnect the line to the valve block.

Detector and HPLC Maintenance

Column Maintenance

Guidelines for Column Maintenance and Replacement

Note: Replace the guard column once a week. Instructions for replacing the guard column are included in the "Column Replacement Requirements" on page 7-21.

If...	Then...
metal contamination is suspected	wash the column with phosphate. Follow the procedure "Washing the 140 Pump and Column with Phosphate" on page 7-25.
1. The chromatography shows consistently broad peaks 2. There is poor separation 3. There are tailing peaks These conditions are not improved by: • Adjusting the composition of solvent A3 • Preparing fresh solvents	replace the column.
the new column dramatically improves the separation	discard the old column. Follow the procedures "Removing the Column" and "Installing the New Column" presented in this subsection.

Column Replacement Requirements

The following items are required to replace the column:

- PTH Column (P/N 401882)
- Guard Column (P/N 401883)

Replacing the Column

Replacing the column involves three procedures:

- Removing the old column
- Installing the new column
- Recording the column change

CAUTION Handle columns carefully. Damaged columns may leak and must be replaced. Do not scratch or dent the column ends. Dropping or bumping the column can irreversibly damage the consistency of the packed bed, thus impairing separation efficiency.

Removing the Column

To remove the old column:

1. Press **Stop** on the front panel of the 140 pump.
2. Press **Manual** to enter manual mode on the 140 pump.
3. If the column is to be reused within a short period of time, flush the column with 90%B for 5 min at a flow rate of 60 µL/min.
4. Change the flow rate to 10 µL/min and the solvent composition to 70 %B.

5. Remove the top portion of the column oven and oven insert to expose the column.
6. Unscrew the guard column and outlet line from the column.
7. Remove the old column.
8. Unscrew the old guard column from the column inlet line.

Installing the New Column

To install the new column:

1. Write the date on the label of the new column, and record the column serial number for later use.
2. Connect the new guard column to the inlet line.
3. Wait until liquid starts flowing out the guard column before proceeding to step 4. Make sure the guard column is functioning properly before you install the new column.

   Loosen the line into the guard column.
4. Connect the guard column to the PTH column inlet port. Then:

   a. Retighten the line into the guard column.

   b. Place the column in the column oven.
5. Wait until the pressure stabilizes and liquid begins coming out of the column before proceeding to step 6.

   IMPORTANT! If you do not wait until liquid passes through the column, you run the risk of air bubbles later getting trapped in the flowcell.
6. Connect the outlet line to the column outlet port.
7. Cover the column with the oven insert and top cover.
8. Go to Start Run screen. Select:

   a. A cartridge

   b. The Fast Column Break-In method for 15 cycles

Recording the Column Change

To record the column change:

1. On the computer, select the Bottle Change dialog box from the dialog box pop-up menu.
2. Click PTH Column in the list of chemicals.
3. Enter the serial number of the new column in the Lot Number box.
4. Open the File menu, and select Save Chemical.
5. Select the Start Run dialog box from the dialog box pop-up menu.

Pump Maintenance Recommendations

Guidelines for 140 Pump

Follow the guidelines below to maintain the 140 pump:

- Replace the seals on the piston and head every 6 months.
- Clean and inspect each cylinder for damage every time the piston seals are replaced. If a cylinder is damaged, it must be replaced.
- Replace the rotor seals after every 3 to 12 months of continuous use.

Refer to the 140 pump user's manual for instructions on replacing the seals.

If...	Then...
you suspect metal contamination in the HPLC components of the system	wash the 140 pump and column with phosphate. See "Washing the 140 Pump and Column with Phosphate" on page 7-25.

Changing Solvents and Purging the 140 Pump

Overview

Changing solvents involves:

- Changing the solvent bottles
- Purging the 140 pump
- Running the 140 pump to equilibrate the column

Purging the 140 pump rapidly expels solvents and trapped gases from the pump's syringes. The pump is equipped with an automatic purge valve to divert the flow of solvent to waste.

Every time a solvent is changed, equilibrate the column with the new solvent(s) until the baseline is stable before sequencing or evaluating a separation. Refer to the *ABI 140 D Micrograd ient Delivery System User's Manual* (P/N 903586) for additional information on changing solvents and purging the pump.

CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death. Wear appropriate protective eyewear, clothing, and gloves.

Changing Solvents

Changing solvents A3 and B2 is performed via the 140 pump control panel.

The keys F1, F2, F3, and F4 are referred to as soft keys, and are followed by the > symbol (PURGE> for example).

The prompts for which you must enter values are shown in all capitals (for example, NUMBER OF PURGES). For more information on this procedure and the 140 pump control panel, refer to the *ABI 140 Micrograd ient Delivery System User's Manual*.

Removing the Old Solvent Bottle

To remove the old solvent bottle:

1. Remove the old solvent bottle(s).
2. Check the solvent lines for obstructions or salt deposits. If the lines are not clear, clean or replace them.
3. Check all fittings for salt deposits or indications of leakage. Clean or replace as necessary.
4. From the Ready Screen (Main Menu) on the 140 pump control panel, press the PURGE> soft key to display the Purge screen. An example of a 140 pump control panel is shown below.

140D	**x.xx cLC**	**FILL>**
PRESS	**EVENTS:0000**	**PURGE>**
CAP A	**CAP B**	**VALVE>**
		UTILITY>

5. Use the arrow keys and numeric keypad to enter the percentage of the syringe to empty and refill:
   a. Enter 2,500 for the PURGE RATE. This is the rate in µL/min at which the cylinders empty.
   b. Move the cursor to the SYRINGE prompt.
   c. Use the Prev./Next keys to select **BOTH**.
   d. Move the cursor to NUMBER OF PURGES, and enter **7**.
   e. Move the cursor to PERCENT OF SYRINGE, and enter **20** or more. (This is the percent of the syringe to empty, refill, and empty again.)

PURGE RATE? 2,500		**BEGIN>**
SYRINGE? BOTH	**# OF PURGES? 7**	
% OF SYRINGE? 20.0	**PURGE NO.**	

Purging the 140 Pump

To purge the 140 pump:

1. Prepare the pump as follows:
   a. Place the solvent inlet line into the new bottle.
   b. Attach the cap.
   c. Place the bottle in the bottle holder.
   d. Repeat for each new bottle.
2. Press the **BEGIN**> soft key to start the purge procedure. The 140 pump and lines are rinsed with fresh solvent. Any air bubbles in the system are removed as well.

3. The status of the procedure is displayed along the bottom of the screen on the 140 pump. To stop the purge procedure, press the **Stop** key.
4. Press the **Manual** key to enter manual mode and display the Manual Status screen. The syringes will fill with new solvent.
5. Press the **FLOW**> soft key. Type **40** to change the flow rate to 40 µL/min. Then press the **Enter** key.
6. Press the %B> soft key, and type **50** to change the solvent composition to 50 %B. Then press the Enter key.
7. Press the PRESS> soft key, and type **3500** to change the maximum operating pressure to 3500 psi. Then press the **Enter** key.
8. Allow the 140 pump to flow at this rate and composition for 10 min to equilibrate the column.
9. Run at least four Flask Standard cLC cycles to check PTH-amino acid separation efficiency and reproducibility before sequencing an unknown sample. If the separation is essentially the same as with the old buffers, begin sequencing.

IMPORTANT! If the separation changes significantly with the new buffers, you may need to optimize the separation. Compare and evaluate the results of the last two cycles to determine if optimization is required. If so, follow the guidelines listed under "Procise PTH-Amino Acid Separation" on page 4-14 in Chapter 4, "Optimizing Sequencer Processes."

Washing the 140 Pump and Column with Phosphate

Use this procedure to clean the entire pumping system if metal contamination is suspected.

Items Required

The following items are required to clean the pumping system:

- Sodium phosphate monobasic or sodium dihydrogen phosphate (NaH_2PO_4). Potassium phosphate monobasic (KH_2PO_4) can be used as a substitute.
- HPLC-grade water
- 500-mL glass beaker

Preparing the Wash Solution

Prepare a 0.1 M solution of sodium phosphate (approximately pH 5.0).

CAUTION The pH of the solution must not exceed 7.0. Ensure that sodium phosphate monobasic is used; otherwise, the pH may be too high.

To prepare the wash solution:

1. Place 3.45 g of sodium phosphate monobasic (NaH_2PO_4) into a 500-mL beaker.
2. Add 250 mL of HPLC-grade water and mix until thoroughly dissolved.

   CAUTION Sodium phosphate will precipitate in acetonitrile. To prevent severe damage to the pumping system, do not allow these two chemicals to mix in the pumping system at any time.

Washing the 140 Pump/Column

WARNING CHEMICAL HAZARD. B2 (12% isopropanol and acetonitrile) is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To wash the 140 pump and the column:

1. Remove the transfer lines from solvents A and B.
2. Purge the 140 pump once at 100%, using the default flow rate.
3. Place the solvent transfer lines into deionized water.
   a. Purge three times.
   b. Press Manual on the 140 pump control panel, and free run the pump at 50 µL/min, 50% B for 15 min.

   Note: This would avoid any salt precipitation in the solvent B line.
4. Place the solvent transfer lines into the phosphate buffer.
   a. Purge three times at 100%, using the default flow rate.
   b. Press **Manual** on the 140 pump control panel, and free run the pump at 50 µL/min, 50% B for 45 to 60 min.
5. Repeat the deionized water purge in step 3.
6. Press Manual on the 140 pump control panel, and free run the pump at 50 µL/min, 50% B for 45 to 60 min.
7. Place the solvent transfer lines back into the respective solvents.
8. Perform one purge at 100%.

Minimizing Further Metal Contamination

To minimize further metal contamination:

1. To minimize the possibility of further metal contamination, add Sodium phosphate monobasic (NaH_2PO_4) or Potassium phosphate (KH_2PO_4) to solvent A.
2. Mix until completely dissolved. The final concentration of phosphate should be 100 µM.

   Note: The addition of phosphate may slightly increase the retention time of aspartic acid and glutamic acid.

Dynamic Pressure Monitoring

Overview

Use this procedure to:

- Monitor the pressure of the 140 pump during a run
- Test the cylinders in the 140 pump

Note: A dual-channel chart recorder is required for this procedure.

Monitoring the Dynamic Pressure

To monitor the dynamic pressure:

1. From the 140 pump Configuration menu, set the Channel and Scale.
   a. Set the D/A channel to A.
   b. Set the scale to 3.

   Note: A scale of 3 will ensure that the pressure trace remains on scale throughout the run (0 to 2040 psi). For a more sensitive response, a scale of less than 3 can be used. In this case, the pressure trace will autozero at several points during the gradient.
2. Connect the chart recorder to the UV detector.
   a. Connect the first channel of the chart recorder to the REC output on the back of the UV detector.
   b. Set the sensitivity for this channel to10 mV full scale.
3. Connect the second chart recorder channel.
   a. Connect the red and black input terminals of the second chart recorder channel to the + and – pressure terminals on the back of the 140 pump (see the figure below).
   b. Set the sensitivity for this channel to 1 V full scale.

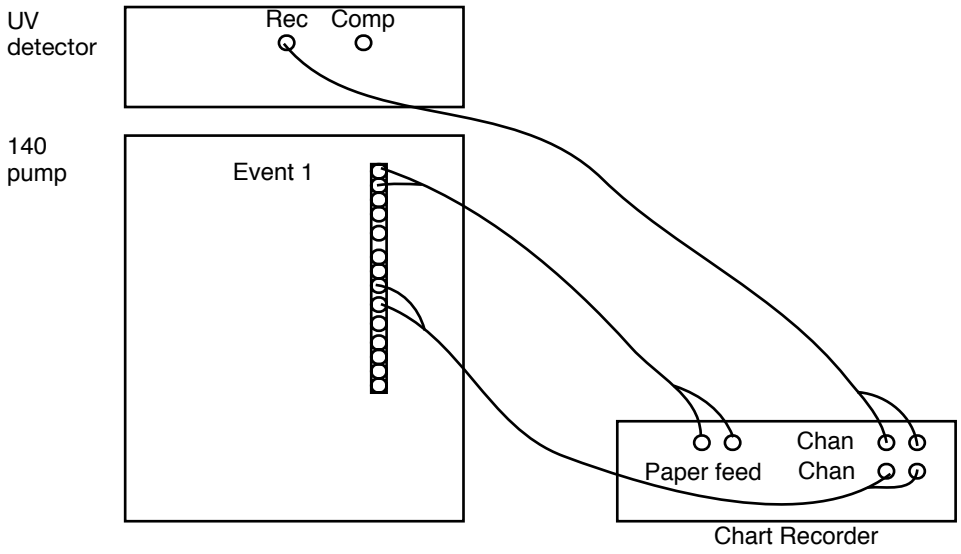


Figure 7-4 Chart recorder cable connections

4. Connect the chart recorder external paper feed input to the Event 1 terminals on the back of the 140 pump.
5. Position both pens using the chart recorder zero controls.
6. Start your run.

Interpreting the Results

The profile of the pressure trace will be gradient specific, but should be consistent from run to run. As shown in Figure 7-5, Channel 1 shows a trace of the chromatogram. Channel 2 shows the corresponding pressure variation during the gradient.

Peaks that go negative indicate a sudden loss of pressure. This could be due to a scratched cylinder. Such a pressure drop would be consistent with a variation in retention time.

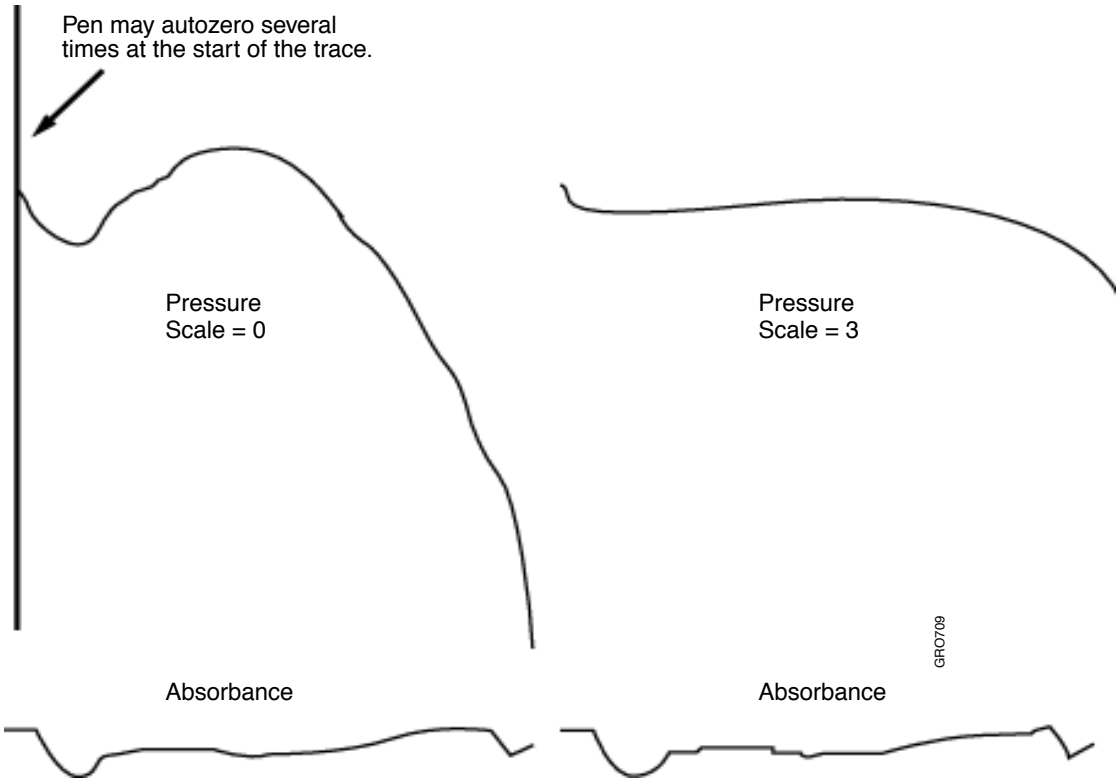


Figure 7-5 Dynamic pressure monitoring

Maintaining the Detector

When to Replace the Lamp

Replace the lamp after every 1500 to 2000 hours of normal use. Refer to the UV detector user's manual for instructions on how to test and replace the lamp.

ULTRAVIOLET LIGHT HAZARD. Exposure to ultraviolet radiation can cause blindness or permanent eye damage. To prevent eye injury, adjust the detector sensitivity from the ultraviolet to the visible range (500 nm) before beginning any detector maintenance procedures. Always wear protective UV-absorbing glasses when looking into the detector.

PHYSICAL INJURY HAZARD. The lamp can become very hot while in use. Turn off the power to the lamp and allow it to cool before removing it from the fixture. Always wear heat-resistant gloves when handling the lamp.

Removing Air Bubbles From the Flowcell

To remove air bubbles from the flowcell:

1. Run 90% solvent B through the flowcell at 60 µL/min.
2. If this does not dislodge the bubbles, flush the flowcell with methanol or isopropyl alcohol.

   See "Removing the Flowcell" on page 7-30 for further instructions.

Guidelines - Cleaning the Flowcell

Cleaning the flowcell can be helpful if:

- Bubbles are still present after running 90% solvent B through the flowcell at 60 µL/min
- Severe drift suggests that contamination is leaching from the flowcell windows

Items Required

The following items are required to flush the flowcell:

- Long flat-blade screwdriver
- Two 1/4-in. wrenches
- Methanol or isopropyl alcohol
- Protective gloves
- HPLC-grade water
- 5–10 mL disposable syringe

Note: For most disposable syringes, the luer adapter can be screwed directly into the flowcell adapter. If this is not the case, make an adapter tube.

CHEMICAL HAZARD. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CHEMICAL HAZARD. **Isopropanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. Exposure may cause central nervous system effects such as drowsiness, dizziness, and headache. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Removing the Flowcell

To prepare for flushing the flowcell, locate chemical-resistant gloves and become aware of the hazards which may be presented during this process.

WARNING **CHEMICAL HAZARD.** Wear chemical-resistant gloves when handling methanol. Contact with skin can cause irritation. Absorption through the skin is harmful. Refer to Chapter 1, Safety (Chemical Summary Table), for further information.

To remove the flowcell:

1. Loosen the knurled captive screw in the upper left hand corner of the rear of the detector.
2. Open the front panel of the UV detector and loosen the knurled captive screw that holds the front panel in place.
3. Slide the detector out of the housing until the top of the detector head is accessible. Open the detector head door.
4. Loosen, but do not remove, the clamping screw (located to the right of the flowcell).
5. Remove the flowcell, complete with inlet and outlet tubing.
6. Disconnect the flowcell tubing.

   IMPORTANT! When loosening or tightening the flowcell bushings, always use a second wrench to prevent the flowcell adaptor from turning.

Flushing the Flowcell

To flush the flowcell:

1. Load the syringe with 5 mL of HPLC-grade water.
2. Flush the flowcell with the water.
3. Load the syringe with 5 mL of methanol or isopropyl alcohol.
4. Flush the flowcell with the methanol or isopropyl alcohol.
5. If this procedure does not remove the trapped material, disassemble the flowcell, clean it, reassemble it, and test it.

Reinstalling the Flowcell

To reinstall the flowcell:

1. Reconnect the inlet and outlet tubing to the flowcell. Do not overtighten; the walls of the adaptor are thin and easily damaged.

   IMPORTANT! When loosening or tightening the flowcell bushings, always use a second wrench to prevent the flowcell adaptor from turning.
2. Push the flowcell back into the clamp so that the body of the flowcell is flush with the clamp, and the inlet tube is to the bottom. If necessary, open the clamp with the screwdriver.
3. Keeping the flowcell loose in the clamp, rotate it so that the outlet tubing is approximately 45° to the left of vertical, and then clamp the flowcell in place. This orientation prevents the tubing from becoming kinked.

4. Route the inlet and outlet tubing through the slot in the detector head top plate. If the plate is not slotted, route the tubing so it sits in the recesses.
5. Tape the tubing in place.

Replacing the Lamp in the UV Detector

Items Required

The following items are required for replacing the lamp:

- New UV lamp (P/N 2900-0484)
- Phillips screwdriver
- UV-protective safety glasses

Replacing the Lamp

ULTRAVIOLET RADIATION HAZARD. Wear UV-protective glasses when performing this procedure.

HOT COMPONENT. Allow the lamp to cool before you touch it.

To replace the lamp:

1. Turn off and unplug the UV detector, and allow the lamp to cool completely.
2. Release the back panel catch by removing the screws.
3. The lamp is held in place by a spring and catch. The catch is located just above the lamp. Using your fingers or a screwdriver, unhook the catch by pushing it forward and slightly upward.
4. Unplug the lamp, and pull it horizontally off the locating pins.
5. Install the new lamp over the two locating pins, and secure the retaining spring.
6. Plug in the lamp.
7. Close the back panel, and power up the detector.

Testing the Dry Cell

Overview

If noise, spikes, drift, or stepping are evident on the baseline, use this procedure to test the integrity of the following:

- Line voltage stability
- UV detector electronics and lamp
- Signal cable between the detector and the protein sequencer
- PROCISE 24-bit A/D convertor

Items Required

The following items are required to test the dry cell:

- Strip chart recorder and signal cable (if available)
- Dry cell aperture (normally taped inside front compartment of detector)

Setting Up the Dry Cell Test

To set up the dry cell test:

1. Replace the flowcell with the spare dry cell aperture.

   Note: Do not disconnect the flowcell from the plumbing.

2. Set the wavelength to 238 nm, the rise time to 1.0 sec, and the range to 0.001.
3. Connect the REC output to the chart recorder. Leave the COMP output connected to the protein sequencer.
4. Set the chart recorder scale to 10 mV full scale and the speed to 2 mm/min.
5. To collect data, select the Start Run dialog box.
6. Configure a run.
   a. Set the Run Order for Cartridge A to **1**.
   b. Enter a unique file name for the run.
   c. Select **Run Gradient cLC** from the Method pop-up menu.

Running the Dry Cell Test

To run the dry cell test:

1. Click **Start Run**.
2. As soon as the Init Sensor procedure starts, jump to the End step.
3. Click **Next Step** when the Flask cycle begins.
4. When the 140 pump starts to run, jump to step 5, Start Gradient.
5. If you do not want the 140 pump to run, press the Stop key on the front of the 140 pump.
6. If a Collecting window is not displayed, ensure that the SequencePro™ Data Analysis Application software is launched.

Interpreting the Test Results

- Noise should be no greater than 2 x 10^{-5} AU.
- 20-µAU peak-to-peak (as read SequencePro software).
- 0.2-mV peak-to-peak (on the chart recorder).
- Drift should be no greater than 1 x 10^{-4} AU/hour after warm-up.
- The baseline should be free of spikes and steps (apart from the initial autozero).

If...	Then...
the Sequence Pro software and chart recorder trace has excessive noise, steps, or spikes	suspect the: 1. UV lamp 2. Detector electronics 3. Line voltage
only the SequencePro data is affected	suspect the: 1. COMP output 2. Signal cable from the UV detector to the protein sequencer 3. Protein sequencer I/O PCB 4. Protein sequencer power supply

Visually Testing the UV Detector Wavelength

Overview

Visually testing the UV detector wavelength is not an accurate wavelength test. However, it can reveal gross errors that affect sequencing results.

WARNING **ULTRAVIOLET RADIATION HAZARD.** To avoid eye injury, always wear UV-protective goggles when performing this procedure.

Testing the UV Detector Wavelength

To test the UV detector wavelength:

1. Look at the detector display. If it reads: ENTER Wavelength (190 to 700 nm) go to step 7.
2. Press the F7 (CNFG) key.
3. When the next menu appears, press the F1 (INST) key.
4. Press the F1 (LAMP) key.
5. Press the F5 (700) key.
6. Press the return key three times to return to the Main menu.
7. Press 656, then Enter.

   Wait for the display to indicate the wavelength setting has reached 656 nm.
8. Open the Detector Head door, and observe the light emitted from the sample cell and reference cells.

   Both lights should be a bright red color.
9. If the color is not bright red, then either:
   - The wavelength is incorrect
   - The lamp is not lit

   Make repairs or adjustments, as appropriate.

Troubleshooting Guide A

This chapter describes issues that you may encounter when working with the PROCISE® Protein Sequencing Systems.

This appendix covers:

Troubleshooting Table

Overview

Table A-1 is designed to help you troubleshoot most of the problems you may encounter while using the PROCISE cLC protein sequencing system.

The information in the table is arranged by category as follows:

- Chemistry and chromatography problems
- Event log error messages
- Software and communication problems
- Pump and detector error messages

Each category contains subcategories, followed by a brief description of the symptoms you might encounter.

To use this table, look for the category and the symptom you are experiencing. The page number in the right-hand column corresponds to a description of the possible cause(s) and recommended action(s) for that particular problem.

Table A-1 Troubleshooting Table

Category	Symptom	Page
Chemistry and Chromatography Problems		
Baseline Disturbances and Anomalies	1. Baseline Noise, (High Frequency)	A-6
	2. Baseline Stepping	A-7
	3. Baseline Cycling (Wavy Baseline)	A-8
	4. Baseline Spikes	A-9
	5. Baseline Noise (Medium Frequency)	A-10
	6. Baseline Sloping	A-12
	7. Humps or Dips on Baseline	A-14
	8. Baseline Deflection (Consistent)	A-15
	9. Baseline Deflection (Random, Off Scale)	A-15
	10. Unexpected Peaks	A-16
Missing Peaks	11. Flat Baseline, No Injection	A-17
	12. Injection, No Peaks, No Errors.	A-17
	13. Injection, No Peaks, Sensor Error(s)	A-18
	14. Injection, No Peaks, Baseline Dip	A-19
	15. Peaks Missing or Broad	A-19

Table A-1 Troubleshooting Table *(continued)*

Category	Symptom	Page
Poor Recovery, Standard Chromatogram	16. Peaks Short and Broad	A-20
	17. Peaks Short	A-20
	18. Peak Heights Variable	A-20
	19. Low Lysine	A-20
	20. Low Lysine (and PE Cysteine)	A-21
Poor Recovery of Residue Amino Acids, N-terminal, N-terminal	21. Low Serine and Threonine	A-21
	22. Low Asparagine or Glutamine	A-22
	23. Low Glycine	A-22
	24. Low Histidine and Arginine	A-23
Low Repetitive Yield (RY), N-terminal (RY), N-terminal	25. High Lag	A-23
	26. No Lag	A-24
Low Repetitive Yield (RY), N-terminal, C-terminal	27. Low Artifacts, Sample Loop Full Errors	A-24
	28. Normal Artifacts, High Lag	A-24
	29. Low Artifacts, High Lag	A-25
	30. Low Artifacts	A-26
Low Initial Yield, C-terminal	31. Low Artifacts, Sample Loop Full Errors	A-26
	32. Normal Artifacts, Normal Repetitive Yield	A-26
	33. Normal Artifacts, Low Repetitive Yield, High Lag	A-27
Artifact Peaks – N-Terminal	34. High Aniline	A-28
	35. High Oxidized DTT	A-29
Retention Time Problems	36. Variable Retention Time	A-29
	37. Most Peaks Miscalled	A-30
	38. Early Peaks Misidentified	A-31
	39. Shifting Retention Time	A-31
	40. Delayed Retention Time	A-31
Event Log Error Messages		
Cartridge, Flask Load, and Transfer Fluid Sensor Error Messages	41. Fluid Not Detected, No Wet Counts	A-32
	42. Fluid Not Detected, Intermittent Wet Count	A-35

Table A-1 Troubleshooting Table *(continued)*

Category	Symptom	Page
Injector Sample Loop Fluid Sensor Errors	43. Injection, No Peaks, Sensor Error(s)	A-37
	44. Partial Injection, Small Peaks, Sensor Error	A-38
	45. No Injection, No Peaks, Sensor Error(s)	A-38
Other Event Log Error Messages	46. Argon Tank Pressure Too Low	A-40
	47. Cannot Reach Set Temperature	A-40
	48. Communication With HPLC Lost	A-40
	49. Event Buffer Overrun	A-41
	50. Injector Position Error, Sensor Errors	A-42
	51. Insufficient Memory	A-42
	52. Invalid Sensor Dry Reading	A-44
	53. Power Failure	A-44
	54. Vacuum Assist Activating	A-45
Leak Test Error Messages	55. Actual Pressure > 5.5 psi.	A-45
	56. Leak Tests Fail	A-46
	57. Vent Test Fails	A-46
	58. Flask Vent Test Fails	A-46
	59. Leak Test Fails, Pressure High	A-47
	60. Cartridge Leak Test Fails	A-47

Table A-1 Troubleshooting Table *(continued)*

Category	Symptom	Page
Software and Communication Problems		
Communication Errors	61. Communication Lost Between Computer and Protein Sequencer	A-48
Pump and Detector Error Messages		
UV Detector Errors	62. Beeping UV Detector	A-49

Chemistry and Chromatography Problems

Baseline Disturbances and Anomalies

1. Baseline Noise, (High Frequency)

Symptom: Baseline Disturbances

Electronic noise from the UV detector can cause baseline disturbances similar to high frequency noise. Flow and mixing problems produce a slower baseline response.

Note: High frequency noise is always present at some level.

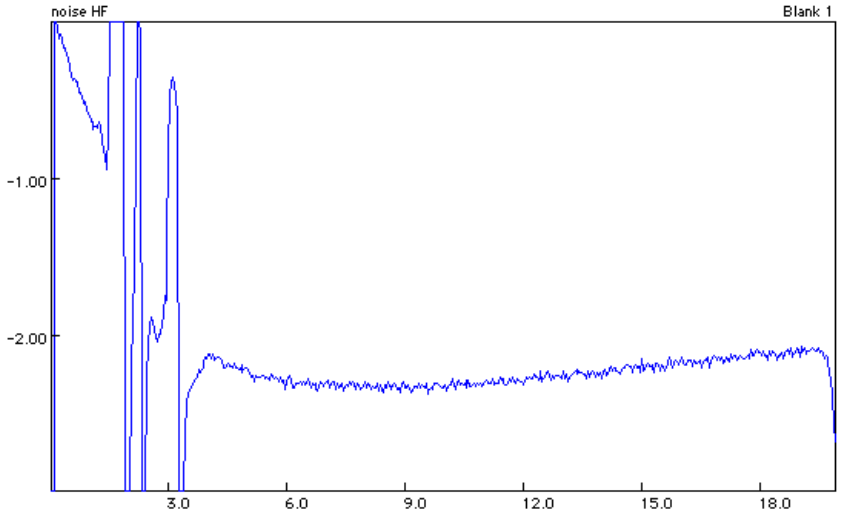


Figure A-1 High-frequency noise (the scale of the diagram has been increased to exaggerate the noise)

Observation	Possible Cause	Recommended Action
Baseline noise, (High Frequency)	Line voltage instability, detector electronics, signal cable or PROCISE 24-bit A/D convertor.	Perform the dry cell test on page 7-32 in Chapter 7, “Maintenance.” Isolate the problem, and fix as recommended.
	The lamp in the UV detector has exceeded its recommended life span (>1000 h). As the UV lamp ages, lamp energy is reduced, and electronic noise is amplified.	Replace the lamp. Refer to Chapter 7 “Replacing the Lamp” on page 7-31 for replacement instructions.
	The wavelength may be set lower than 269 nm. The baseline profile may also change due to different absorbance characteristics of the HPLC solvents.	Set the wavelength to 269 nm.
	Rise time on the detector is not set to 1.0 sec	Set the rise time to 1 sec.
Baseline noise, (High Frequency). Not all chromatograms or sections of the baseline are affected to the same extent.	A small air bubble is trapped in the flow cell, and is interfering with the UV transmission. The noise tends to come and go throughout the cycle and the run. If not all chromatograms or sections of the baseline are affected to the same extent, an air bubble is probably in the flowcell.	The back-pressure PEEK tubing is incorrectly installed onto the flowcell outlet line. Reinstall the back-pressure PEEK tubing.
		There are leaks before or after the flowcell. Repair the leaks.
		Questionable condition of the flowcell. Clean the flowcell by following the flushing procedure listed on page 7-30 in Chapter 7, “Maintenance.”

2. Baseline Stepping

Symptom: Intermittent Stepping of Baseline in Chromatograms.

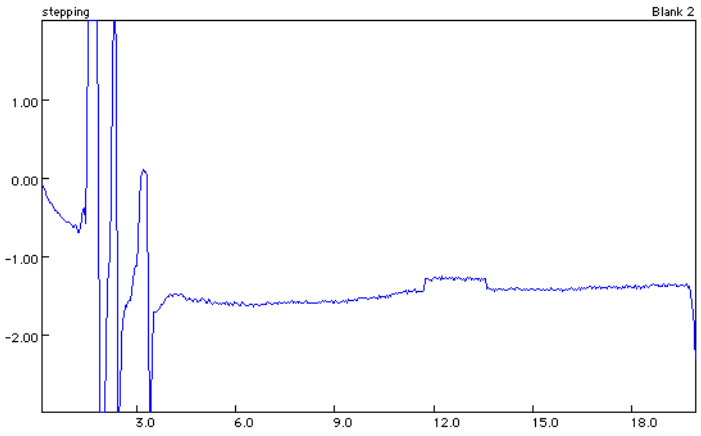


Figure A-2 Baseline stepping

Observation	Possible Cause	Recommended Action
Baseline stepping	Aging ultraviolet (UV) lamp exceeding its recommended life span (>1000 h). The electrodes burn and the arc becomes less stable. This results in a step on the baseline (Figure A-2).	Replace the UV lamp. Refer to Chapter 7, “Replacing the Lamp” on page 7-31 for replacement instructions.
	Line voltage instability, detector electronics, signal cable, PROCISE 24-bit A/D converter	Perform the dry cell test on page 7-32 in Chapter 7, “Maintenance.” Repair any problems found as recommended.

3. Baseline Cycling (Wavy Baseline)

Symptom: Cycling of Baseline (low frequency).

Observation	Possible Cause	Recommended Action
Baseline cycling (wavy baseline)	Slow current variation. Interference from the environment is not effectively screened out. Incorrect or poor electrical grounding could cause interference.	Plug the system into a different, stable circuit. Perform the dry cell test on page 7-32 in Chapter 7, “Maintenance.” Repair any problems found as recommended.
	All system instruments are not plugged into the same power source.	Plug all instruments into the same power source. For example, if a universal power supply (UPS) is being used, all of the instruments should be plugged into it.
	The detector fan in the UV lamp compartment is not functioning or the detector is exposed to significant temperature variations.	Contact your local tech support or field service representative for assistance.
	The signal cable shielding is not grounded correctly.	Ground the signal cable shielding correctly.
	The UV lamp in the UV detector has exceeded its recommended life span (>1000 h).	Replace the UV lamp. Refer to page 7-31 in Chapter 7, “Maintenance.” for replacement instructions.

4. Baseline Spikes

Symptom: Baseline Spikes.

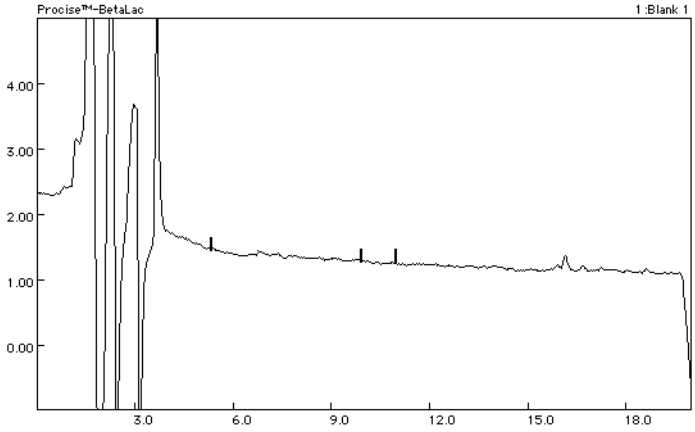


Figure A-3 Spikes on baseline

Observation	Possible Cause	Recommended Action
Baseline spikes. The spikes will be seen only when the 140 pump is running.	There are line voltage disturbances (Figure A-3).	Plug all instruments into the same power source. For example, if a universal power supply (UPS) is being used, all of the instruments should be plugged into it.
		Plug the system into a different, stable circuit. A dry cell test on page 7-32 in Chapter 7, “Maintenance.” can help reveal power supply disturbances.
	The column is losing column packing material (silica). Indicators of this problem: • A build up of white material in the flowcell • Precipitate at the column outlet	Replace the column, and clean the flowcell by flushing it with 90% B or methanol. Refer to Chapter 7 “Column Replacement Requirements” on page 7-21 for column replacement instructions.
	Air bubble in flowcell.	Set the detector wavelength to 656 nm, and look for an air bubble in flowcell. If there is an air bubble: 1. Increase the solvent composition to 90%B at 50 µL/min. for a 140D pump and 325∫µL/min. for a 140C pump to flush out the bubbles. 2. Check for leakage at the flowcell outlet and repair if necessary.

5. Baseline Noise (Medium Frequency)

Symptom: Noise (medium frequency) on Baseline.

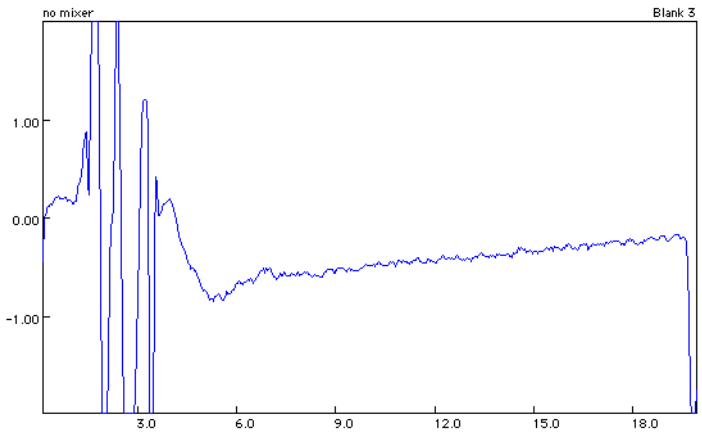


Figure A-4 Dynamic mixer not turning

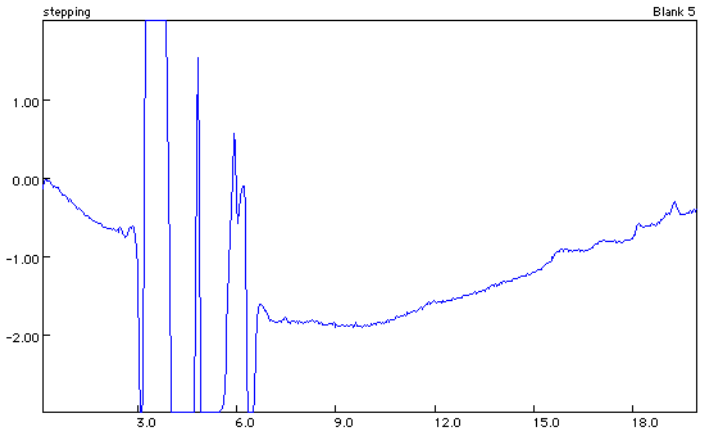


Figure A-5 Leaking pump seal

Observation	Possible Cause	Recommended Action
Baseline noise (Medium Frequency) Stable retention times	Bad fuse Aging lamp	1. Check the 1A fuse in the 140 pump. Replace the fuse if necessary. (Refer to the 140 pump user's manual for instructions on replacing the 1A fuse.) 2. Find out if the UV lamp in the UV detector has exceeded its recommended life span (>1000 h). If so, replace the UV lamp. (Refer to page 7-31 in Chapter 7, "Maintenance." for replacement instructions.)
	Inefficient mixing. A mixing problem is characterized by a dip at the front end of the chromatogram (Figure A-4). Normally, a mixing problem will have less impact on retention time variation.	Contact your local tech support or field service representative for assistance.
Baseline noise (Medium Frequency) Unstable retention times	Leaks	Look and repair leaks at: 1. Pump manifold fittings. 2. All fittings and pump seal leak points. A bad seal can cause a retention time shift and baseline abnormalities (see Figure A-5 on page A-10). If there are no obvious leaks (Figure A-6) then perform the static pressure test. Monitor the system pressure during a run to determine the source of the leak.
	Air in the system	Check for: • Sufficient solvents • Leaking solvent inlet lines or fittings • Leaks at tubing connections Purge pumps several times to eliminate air from the system.

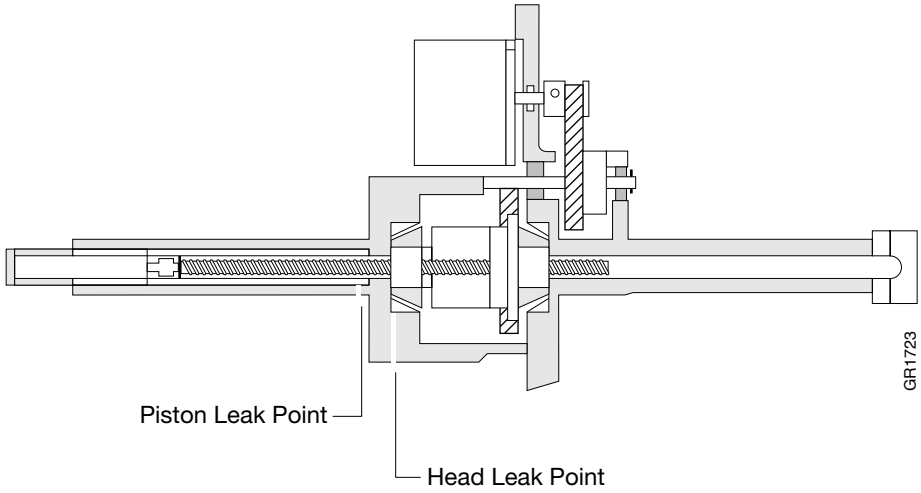


Figure A-6 Pump seal leak points

6. Baseline Sloping

Symptom: Sloping Front End.

Early-eluting amino acids (Figure A-7) can “ride” on a sloping baseline. A hump early in the chromatogram usually indicates the presence of a contaminant in the pumping system. Baseline sloping is common after replacing a system component such as a pump seal and is usually disappears on its own over time.

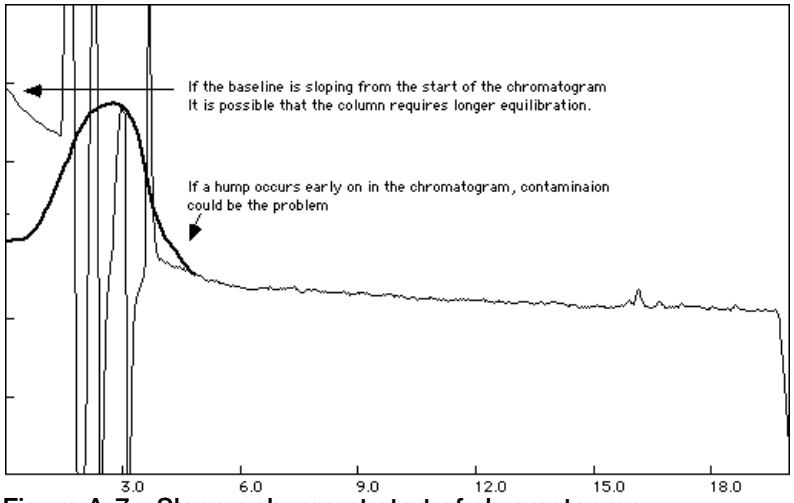


Figure A-7 Slope or hump at start of chromatogram

Observation	Possible Cause	Recommended Action
Baseline front end sloping	UV-absorbing contaminant in solvent A	Wash the column and pumping system. Refer to pages 7-26 in Chapter 7, “Maintenance.” for wash instructions. To minimize the slope, add sodium phosphate monobasic to solvent A.
	Inadequate column equilibration	Increase the equilibration time. (The column equilibration time is the time between the Prepare Pump and Load Injector steps in all Flask cycles.)
	The 140 pump is not configured to “Fill between runs”	Configure the pump to “Fill between runs”: 1. Press **Run** on the front panel of the instrument. 2. Select **Y** for Manual.
	The 140 pump is not configured to continue pumping after completion of the gradient	Configure the pump to continue pumping after completion of the gradient: 1. Press **Run** on the front panel of the instrument, 2. Select **Y** for Manual.

7. Humps or Dips on Baseline

Symptom: Humps or Dips on Baseline.

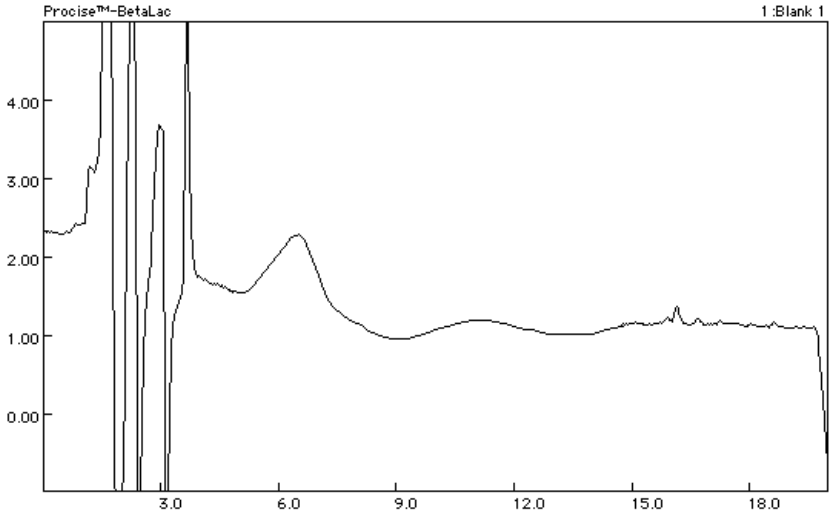


Figure A-8 Hump on baseline

Observation	Possible Cause	Recommended Action
Humps or dips on baseline	Protein sequencer problem	Run the Run Gradient (cLC) method to isolate the HPLC components of the system from the protein sequencer. If the profile is normal then: 1. Clean or replace the flask. 2. Replace R4A and S4. Cleaning instructions are in Chapter 7, “Maintenance.”
	Pumping system problem	Run the Run Gradient (cLC) method to isolate the HPLC components of the system from the protein sequencer. If the profile is not normal then: 1. Replace solvents A and B. 2. Purge the system 3 times. Instructions for changing solvents and purging the 140 pump are in Chapter 7, “Maintenance.”
	The detector flowcell is not flush with the monochromator. Refractive index effects are exaggerated.	Reposition the flowcell.

8. Baseline Deflection (Consistent)

Symptom: Baseline Deflection (Consistent).

Observation	Possible Cause	Recommended Action
Baseline deflection (Consistent in all chromatograms)	Scratched cylinder in the 140 pump	1. Monitor the pressure as the system is running and look for a sudden change in pressure consistent with the deflection on the chromatogram. 2. Remove and inspect the cylinders in the 140 pump. 3. Replace the damaged cylinders. Refer to the 140 pump user's manual for instructions.

9. Baseline Deflection (Random, Off Scale)

Symptom: Baseline Deflection (Random, Off Scale).

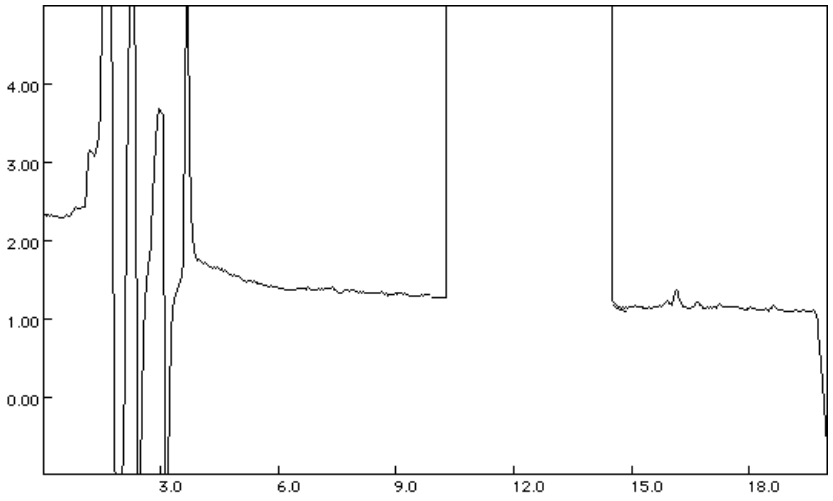


Figure A-9 Air bubble in flowcell

Observation	Possible Cause	Recommended Action
Baseline deflection (random, off scale)	An air bubble is trapped in the flowcell (Figure A-9). Air bubbles tend to occur with high concentrations of aqueous solvent.	Check for air bubbles in the flowcell by: 1. Setting the wavelength to 656 nm 2. Looking into the flowcell noticing if the red light is diffracted 3. Clear the air bubbles by washing out the flowcell by increasing the solvent composition to 90% B at 50 µL/min for a 140D pump and 325ʃµL/min. for a 140C pump to flush out the bubbles.

Observation	Possible Cause	Recommended Action
Baseline deflection (random, off scale)	The back pressure line is not properly installed.	Reinstall the back pressure line.
	Adequate quantities of solvents A and B are not present.	Replenish the solvents.

The Back-Pressure Line (140D pump)

Install a back-pressure line to effectively prevent the formation of bubbles. It is then unnecessary for users to de-gas the solvents.

The back-pressure line consists of 18–24 in. of 0.0025-in. i.d. PEEK tubing.

10. Unexpected Peaks

Symptom: Unexpected Peaks.

Sometimes additional peaks may be in the Blank, Standard and Residue chromatograms. The peaks can appear anywhere after the Injection Artifact.

<table>
<tr><th>Observation</th><th>Possible Cause</th><th>Recommended Action</th></tr>
<tr><td>Unexpected peaks</td><td>A contaminant is present:
In solvents A or B
In the flask reagents
In the flask system
In the pumping system</td><td>Start the Run Gradient cLC method to isolate the HPLC components of the system from the conversion flask.<table><tr><th>If...</th><th>Then...</th></tr><tr><td>the peaks are still present</td><td>Replace solvents A and B.</td></tr><tr><td>are not present</td><td>1. Replace the flask reagents (R4A, S4, and R5).
2. Clean or replace the conversion flask. Cleaning instructions (including removal) are in “Maintaining the Conversion Flask Vial” on page 7-12 or “Replacing the Conversion Flask Vial” on page 7-15.
3. Replace the injector loop.</td></tr></table></td></tr>
</table>

Missing Peaks

11. Flat Baseline, No Injection

Symptom: Flat Baseline, No Injection artifact.

There are no event log errors.Since data collection occurred, and there were no errors in the event log, an injection took place.

Observation	Possible Cause	Recommended Action
Flat baseline, no Injection	The 140 pump	Run the pump in manual mode to verify pressure and flow through the injector column and flow cell.
	The A/D convertor	Monitor detector output using a strip chart recorder.
	The UV detector	Check: 1. The UV lamp status by pressing the UTIL> key on the front panel of the detector. The reference reading should fall between –0.25 and –0.4. 2. If the reading is incorrect then replace the lamp by following the procedure on page 7-31 in Chapter 7, “Maintenance.”

12. Injection, No Peaks, No Errors

Symptom: Injection, No Peaks, No Errors.

There may be some aniline present. There are no event log errors. All liquid deliveries and transfers took place, since there were no fluid sensor errors in the event log.

Observation	Possible Cause	Recommended Action
Injection, no peaks, no errors	No R2 vapor delivery	Perform a bottle leak test on the R2 bottle. Repair any leaks. Refer to the “Cartridge Leak Test Procedure” on page 6-17.
	The R2 Set and Actual pressures are not set to the default values (0.8 psi)	Go to the Pressures & Temperatures dialog box, and click Default to restore the default settings. **Note:** It is normal for the Actual pressure to float a little higher than the Set pressure when there is no R2 delivery.
	The R2 bottle is empty	Replace the bottle.

13. Injection, No Peaks, Sensor Error(s)

Symptom: Injection, No Peaks, Sensor Error(s).

There is a transfer sensor error in the event log.

Observation	Possible Cause	Recommended Action
Injection, no peaks, sensor error(s)	Blockage in the transfer line	Check the transfer line for blockages by running the Sensor and Delivery test (a Flow test). A transfer sensor error indicates a transfer line is blocked. Contact your local tech support or field service representative for
	A cartridge outlet sensor determines that it is sensing liquid when, in fact, it is not. S2 extraction and transfer will not occur.	See if the cartridge lines are crimped. Contact your local tech support or field service representative for assistance.
		Watch a “Deliver S2, cart sensor” function and see liquid reach the cartridge before the “fluid sensed” light turns on. The light turns on as soon as the function is executed. Reinitialize the sensors by running the Init Sensor Procedure in Chapter 6, Tests and Procedures and try again. If a problem still exists: • The cartridge line flushing function is failing. and/or • The sensor is faulty.
	Incorrect pressure	See if regulator #5 is set to 0. A cartridge leak test may have been aborted before the operating pressure was saved. Never abort a leak test. 1. Adjust the pressures. 2. Click **Default** in the Pressures and Temperatures dialog box to restore the default settings.

14. Injection, No Peaks, Baseline Dip

Symptom: Injection, No Peaks, Baseline Dip.

- No other peaks.
- There is an "Injector full" sensor error in the event log.

Note: If an injection artifact is present then some form of injection took place.

Observation	Possible Cause	Recommended Action
Injection, no peaks, baseline dip	An air injection. The sample loop load sensor was incorrectly initialized. or The residual liquid was not completely flushed out of the injection system prior to the Load Injector step. A small dry reading of 29 (0.15 sec) in the sample loop full sensor error means: • The injector was actuated almost immediately after the Load Injector step started. and/or • The injector was actuated before any liquid could reach the sample loop.	Run the Post-Run Valve Block Wash X1-X2 procedure. This procedure is run from the Test dialog box, and is listed under Shutdown procedures.

15. Peaks Missing or Broad

Symptom: Peaks Missing or Broad.

Observation	Possible Cause	Recommended Action
Peaks missing or broad	The pumping system is not delivering an adequate concentration of organic solvent.	Check the cable connection between the inject output of the protein sequencer and the inject input of the 140 pump. Reconnect a loose cable.
		Check if the correct gradient is being used. Choose the correct gradient.
		Look for HPLC system component leaks. Repair any leaks. Refer to the 140 pump user's manual for instructions on detecting and repairing leaks.

Poor Recovery, Standard Chromatogram

16. Peaks Short and Broad

Symptom: Peaks Short and Broad.

Observation	Possible Cause	Recommended Action
Peaks short and broad	The column is losing plate count.	Replace the column. Replacement instructions are on page 7-21 in Chapter 7, “Maintenance.”
	The pumping system is severely contaminated.	Wash the column and pumping system with phosphate. Washing instructions are in “Washing the 140 Pump and Column with Phosphate” on page 7-25.

17. Peaks Short

Symptom: Peaks Short.

Observation	Possible Cause	Recommended Action
Peaks are short	Wrong detector output	Check that the protein sequencer signal cable is plugged into the COMP output on the detector.
	Aged solvents	Replace solvents A and B with fresh solutions, and purge the 140 pump. Instructions are in and “Purging the 140 Pump” on page 7-24.

18. Peak Heights Variable

Symptom: Peak Heights Variable.

Observation	Possible Cause	Recommended Action
Peak heights variable	Liquid is bubbling too much in the flask and flowing through the flask vent valve to waste.	Check to see if the flask bubble pressure is at 1.8 psi. Click **Default** in the Pressures and Temperature dialog box to restore the default settings.

19. Low Lysine

Symptom: Low Lysine.

Lysine is modified:

- PTH-hydroxylysine elutes just after PTH-valine.
- PTH-methyllysine elutes just after PTH-leucine.
- PTH-succinyllysine elutes midway between DMPTU and PTH-alanine.

In general, if the lysine is normal height (taller than leucine) in the PTH-amino acid standard chromatogram but not in the residue cycles, then the HPLC components of the system and solvents are not responsible for lysine degradation. Instead, there may be a problem with the reaction cartridge chemistry or delivery system.

Observation	Possible Cause	Recommended Action
Low lysine	Metal contamination. Lysine is extremely sensitive to metal contamination and the peroxides that can form in solvent A as a result of THF oxidation.	Check the vacuum tubing at the valve block manifold for discoloration. Replace the valve block if tubing is discolored.
		Replace solvent A.
	Contaminated S2. Lysine can be destroyed.	Replace S2.

20. Low Lysine (and PE Cysteine)

Symptom: Low Lysine (and PE Cysteine).

Authentic PTH-cysteine is generally not recovered in sufficient yield to be seen.

Observation	Possible Cause	Recommended Action
Low lysine (and pe cysteine)	Solvent A is contaminated	Replace solvent A.
	The final concentration of phosphate is less than 100 mmol in solvent A	1. Add phosphate to solvent A so that the final concentration is 100 mmol. 2. Wash the column and pumping system with phosphate. Washing instructions are in “Washing the 140 Pump and Column with Phosphate” on page 7-25.

Note: Lysine and cystine are sensitive to metal contamination and the peroxides that can form in solvent A as a result of THF oxidation.

Poor Recovery of Residue Amino Acids, N-terminal

21. Low Serine and Threonine

Symptom: Low Serine and Threonine.

Serine Overview

A significant amount of serine dehydrates during cleavage to form dehydroalanine. Dehydroalanine is very reactive and unstable. The DTT added to R4 reacts with the dehydroalanine derivative and has a stabilizing effect.

This DTT-dehydroanaline derivative, commonly called delta-serine (δS), elutes between PTH-alanine and PTH-tyrosine. It can be used to help identify a serine residue.

Threonine Overview

A significant amount of threonine dehydrates during cleavage to form dehydro-alpha-aminoisobutyric acid. This product reacts with the DTT added to R4 to produce two to four derivatives. These derivatives elute midway between PTH-tyrosine and PTH-proline.

Observation	Possible Cause	Recommended Action
Low serine and threonine	The Pre-conversion Dry step is too long. This is the most critical step for these amino acids: Some liquid must still be present in the flask at the end of the Pre-conversion Dry step. It is better to have too much liquid left, even though this will dilute the R4. Plenty of TFA must be present for conversion to take place.	Shorten the length of Function 236, Pre-conversion Dry. Instructions for modifying functions are on page 5-2 in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients.”

22. Low Asparagine or Glutamine

Symptom: Low Asparagine with High Aspartic Acid or Glutamine with Low Glutamic Acid.

Asparagine and Glutamine Overview

Under typical conversion conditions, approximately 10% of PTH-asparagine and PTH-glutamine are degraded by deamidation to yield PTH-aspartate and PTH-glutamate respectively in the conversion flask.

Observation	Possible Cause	Recommended Action
Low asparagine or glutamine	Severe deamidation	Check sample storage or handling. Make improvements wherever possible.

23. Low Glycine

Symptom: Low Glycine.

Glycine Overview

ATZ-glycine converts to PTH-glycine somewhat slowly. The reaction is only 80–85% complete during normal conversion conditions. The remaining 15–20% elutes as PTC-glycine near the end of the solvent front.

Observation	Possible Cause	Recommended Action
Low glycine	The flask temperature may be too low, or the flask heater may have failed	Check the flask temperature. It should be 64 °C.

24. Low Histidine and Arginine

Symptom: Low Histidine and Arginine.

Observation	Possible Cause	Recommended Action
Low histidine and arginine	Histidine and arginine dried completely on the glass fiber filter. Histidine and arginine are very difficult to extract from glass fiber filters, especially if allowed to dry completely. The most critical step for histidine/arginine recovery is the post-cleavage Dry Cartridge step right before the *Ready Transfer to Flask* step.	Reduce: • The time of the post-cleavage Dry Cartridge step before the Ready Transfer to Flask step: • Reduce it 10 sec at a time, until histidine and arginine recovery is improved. However, if this step is too short, and an excessive amount of TFA (R3) remains, the sample will washout.
	Conversion flask and/or pick-up line is dirty.	Look for presence of white precipitate. Replace conversion flask and/or pick-up line.

Low Repetitive Yield (RY), N-terminal

25. High Lag

Symptom: Low Repetitive Yield, High Lag.

Lag is due to incomplete coupling, or incomplete cleavage of the N-terminal amino acid. Lag is nominally 1.5% of the residue excluding proline in the previous cycle.

Observation	Possible Cause	Recommended Action
Low repetitive yield, high lag	No base (R2g) delivery (but no sensor errors). In Pulsed-liquid cycles, the TFA (R3) is metered by a fluid sensor.	Check the R2 delivery/pressurization path for restrictions by running the R2 Leak Test Bottle Change procedure. If the vent test portion of the procedure fails, there is a blockage in the R2 pressurization path.Use a flowmeter or: 1. Set the R2 pressure to 0.3 psi. 2. Activate Function 11, Del R2g, Cart (top), and allow it to equilibrate for 1 min. The pressure valve clicking frequency should be approximately 2.5 clicks/sec.
	The R3 valve is leaking, and TFA is constantly leaching into the system. This will neutralize the basic environment required for coupling.	Contact your local tech support or field service representative for assistance.

26. No Lag

Symptom: Low Repetitive Yield, No Lag.

Observation	Possible Cause	Recommended Action
Low repetitive yield, no lag	Wash out may have occurred. Since there is no lag, coupling and cleavage are OK. The height of PMTC, DPTU and DPU is reduced when washout occurs.	Check if the S2 pressure is set to 1.7 psi. If not, click Default in the Pressures and Temperatures dialog box to restore the default setting.
		Ensure that the BioBrene™ solution applied to glass-fiber filters is completely dry before precycling.
		Try a new lot of BioBrene solution.

Low Repetitive Yield (RY), C-terminal

27. Low Chemistry Artifacts

Symptom: Low Repetitive Yield, Low Chemistry Artifacts, Sample Loop full Errors in the Event Log.

Observation	Possible Cause	Recommended Action
Low repetitive yield, low chemistry artifacts, sample loop full errors in the event log	Sample bubbling out of the flask because the flask bubble pressure is too high or the flask walls are coated with chemistry by-products.	• Verify that the: – Flask bubble pressure is set to 1.0 psi. – The C5 bottle contains acetonitrile for washing the flask. • Substitute acetone for acetonitrile in the C5 bottle. • Replace the flask vial.

28. Normal Chemistry Artifacts, High Lag

Symptom: Low Repetitive Yield, Normal Chemistry Artifacts, High Lag.

Observation	Possible Cause	Recommended Action
Low repetitive yield, normal chemistry artifacts, high lag	Poor cleavage due to insufficient TFA (C12) delivery	Check the TFA (C12) delivery in the sequencing cycle.

29. Low Chemistry Artifacts, High Lag

Symptom: Low Repetitive Yield, Low Chemistry Artifacts, High Lag.

Observation	Possible Cause	Recommended Action
Low repetitive yield, low chemistry artifacts, high lag	Poor alkylation due to insufficient DIEA (C11) or bromomethylnaphthalene (C8) delivery.	• Check the reagent levels in the C8 and C11 bottles • Run the C8 and C11 bottle leak tests to check for a leak or a restricted check valve. Repair or replace if necessary. • Check for blockage or restriction in the delivery lines or flow paths.

30. Low Repetitive Yield, Low Chemistry Artifacts

Symptom: Low Repetitive Yield, Low Chemistry Artifacts.

Observation	Possible Cause	Recommended Action
Low repetitive yield, low chemistry artifacts	Poor thiohydantoin formation due to insufficient Bu_4NCS (C10) or excessive TFA (C12) delivery.	• Check the reagent levels in the C10 and C12 bottles. Liquid level in the C12 bottle must be below the cartridge delivery line. • Run the C10 bottle leak test to check for a leak or a restricted check valve. Repair or replace if necessary. • Check the TFA (C12) delivery in the sequencing cycle. The delivery, step 47, should be 90 seconds. • Check for the blockage or restriction in the C10 delivery line or flow path.

Low Initial Yield (IY), C-terminal

31. Low Chemistry Artifacts

Symptom: Low Initial Yield, Low Chemistry Artifacts, Sample Loop Full Errors in the Event Log.

Observation	Possible Cause	Recommended Action
Low initial yield, low chemistry artifacts, sample loop full errors in the event log	Sample bubbling out of the flask because flask bubble pressure is too high or flask walls are coated with chemistry by-products.	• Verify that the: – Flask bubble pressure is set to 1.0 psi. – The C5 bottle contains acetonitrile for washing the flask. • Substitute acetone for acetonitrile in the C5 bottle. • Replace the flask vial.

32. Normal Chemistry Artifacts, Normal Repetitive Yield

Low Initial Yield, Normal Chemistry Artifacts, Normal Repetitive Yield.

Observation	Possible Cause	Recommended Action
Low initial yield, normal chemistry artifacts, normal repetitive yield	C-terminal activation is not proceeding efficiently because of a problem with the acetic anhydride or insufficient initial TFA delivery.	• Replace acetic anhydride (C6). acetic anhydride should be changed after three to four weeks on the sequencer. • Check the TFA (C12) deliveries in the activation cycle. Each delivery, steps 40, 57, and 74 should be 40 seconds.

33. Normal Chemistry Artifacts, Low Repetitive Yield, High Lag

Symptom: Low Initial Yield, Normal Chemistry Artifacts, Low Repetitive Yield, High Lag.

Observation	Possible Cause	Recommended Action
Low initial yield, normal chemistry artifacts, low repetitive yield, high lag	Insufficient TFA (C12) delivery.	• Run the TFA (C12) bottle leak test to check for a leak or a restricted check valve. Repair or replace if necessary. • Check for blockage or restriction in the TFA delivery line or flow path.

Artifact Peaks – N-Terminal

34. High Aniline

Symptom: High Aniline.

Expect to see aniline at the sub-pmol level. Aniline elutes between PTH-asparagine and PTH-serine, and can interfere with either derivative if you are working at high sensitivity.

<table>
<tr><th>Observation</th><th>Possible Cause</th><th>Recommended Action</th></tr>
<tr><td rowspan="4">High aniline</td><td>Dead volumes in the reaction plumbing where unreacted PITC is being trapped</td><td>Reinstall the cartridge line, making sure the end of the line is flush with the tip of the ferrule.</td></tr>
<tr><td>The glass blocks may be dirty</td><td>Thoroughly clean the glass cartridge blocks. Cleaning instructions are in Chapter 7, “Maintenance.”</td></tr>
<tr><td>The cartridge line may not be flush with the end of the ferrule if the size of the peak is larger for a particular cartridge</td><td>Reinstall the cartridge line, making sure the end of the line is flush with the tip of the ferrule.</td></tr>
<tr><td>• Slightly dirty or incorrectly adjusted flask pick-up line
• Slightly dirty flask vial
• Insufficient post-conversion dry</td><td>1. Check for build-up of white precipitate in the bottom of the flask vial or in the tip of the pick-up line.
2. Replace vial and pick-up line if build-up is observed.
<table>
<tr><th>If...</th><th>Then...</th></tr>
<tr><td>no precipitate is observed</td><td>check that the end of the pick-up line is within 1-2 mm from the bottom of the flask vial. Adjust if necessary.</td></tr>
<tr><td>the pick-up line is adjusted correctly</td><td>increase the time of Function 237, Post-conversion Dry. As a general rule, after the flask is visibly dry, continue to dry an additional 180 sec. *</td></tr>
</table>
*. Increasing the time of this function will not effect amino acid recovery, since the amino acids are in the stable PTH form at this time.
Instructions for modifying functions are in “Creating and Modifying Functions” on page 5-2.</td></tr>
</table>

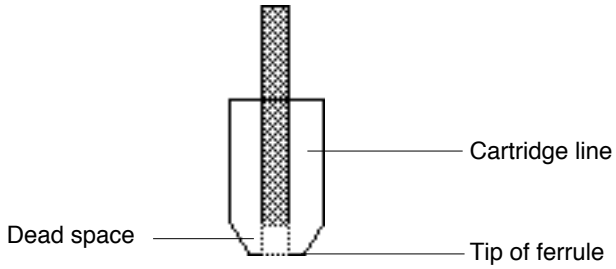


Figure A-10 Incorrectly installed cartridge ferrule

35. High Oxidized DTT

Symptom: High Oxidized DTT.

Observation	Possible Cause	Recommended Action
High oxidized DTT	R4 is bad. DTT is added to both R4 (25% TFA in water) and R5 (acetonitrile) during the manufacturing process. DTT is an oxygen scavenger, and the oxidized DTT reaction product appears as a peak immediately after the injection artifact.	Replace the R4 if the DTT peak is so high that PTH-aspartate rides on its shoulder.

Retention Time Problems

36. Variable Retention Time

Symptom: General Retention Time Instability During The Run.

Observation	Possible Cause	Recommended Action
Variable retention time	Leak in the pumping system, reducing the flowrate	1. Visually inspect all fittings for leaks and repair accordingly. 2. Check the pump seal leak points (Figure A-6 on page A-12) for liquid. Repair any leaks found. 3. Perform the static pressure test, and repair any leaks accordingly. Procedures are located in the 140 pump user's manual.

37. Most Peaks Miscalled

Symptom: Most Peaks Miscalled Even Though Peak Retention Times Reasonably Stable.

Observation	Possible Cause	Recommended Action
Most peaks miscalled	A fluctuating laboratory temperature	Stabilize your laboratory temperature.
	A non-suitable reference peak was chosen	Change your reference peak.

Reference peak guidelines:

A reference peak enables the SequencePro™ Data Analysis Application software to compensate for similar shifts of all peaks in the same direction. The reference peak must be:

- Present in all residue (sequencing) cycles and the PTH-amino acid standard cycle
- Well-resolved from amino acid peaks (± 0.25 min)
- The largest peak, if part of a group of non-amino acid peaks

PTH-standard mixture peaks:

The PTH-standard mixture currently includes four peaks that are not amino acids:

- DMPTU
- DPTU
- DPU
- PMTC

The suitability of these and other reference peaks is described below:

Reference Peak:	Suitability:	Reason:
DMPTU	Not suitable	DMPTU is not produced as a by-product of the N-methylpiperidine chemistry
DPTU	Suitable	DPTU is only useful as a reference peak if it appears larger than the PMTC peak in the residue cycles.
PMTC	Suitable	PMTC is normally a larger peak than the DPTU in the residue cycles, and is an ideal candidate for a reference peak.
DPU	Some suitability	DPU is the oxidation product of DPTU. It can be used as a reference peak if an adequate amount is generated in each cycle.
Amino Acid	Some suitability	A suitable Amino Acid can also be used as a reference peak if there is significant background in each cycle.
None	Some suitability	If laboratory temperatures are stable and the column has settled down, there may be no need to use a reference peak at all.

38. Early Peaks Misidentified

Symptom: Early Peaks Misidentified.

Observation	Possible Cause	Recommended Action
Early peaks misidentified	TFA injected onto the column because the flask is not completely dry after the Post-conversion dry step If TFA is injected onto the column, it tends to affect the retention times and resolution of early eluting amino acids.	Modify Function 237, Post-conversion Dry, so the flask is completely dry 90–120 sec before completion of this step.

39. Shifting Retention Time

Symptom: Shifting Retention Time.

Observation	Possible Cause	Recommended Action
Shifting retention time	Air sucked into the cylinders due to a leak at the pump inlet manifold	Check the fittings at 140 pump inlet manifold and Rheodyne valve for leaks. Repair any leaks that are found. Refer to the 140 pump user's manual for instructions on detecting and repairing leaks.
	A partially blocked solvent filter	Sonicate the solvent filters in nitric acid.

40. Delayed Retention Time

Symptom: Delayed Retention Time.

```
01/17/1995      21:26:40
When the Load Injector function is finished, the Rheodyne valve must be in the inject position.
```

Figure A-11 Injector Position event log Message

Observation	Possible Cause	Recommended Action
Delayed retention time	There is a software bug in the PROCISE 1.0 firmware. The predicted frequency of occurrence is extremely low (1 in 1500 injections).* The injector failed to turn during the Load Injector step if the message in Figure A-11 appears in the event log. It turned during the subsequent Inject Position step.	1. Check the Event Log for any Sample Loop Load errors. If the Sample Loop Load errors are found, check that the flask vial and pickup line are clean. Replace a dirty line. 2. Run a few standards to verify that the sample loop is loading correctly.

*. Data collection starts as soon as liquid is sensed at the Sample loop load sensor (approximately 10 sec after the Load Injector step begins). However, the gradient does not start until the injector moves to the inject position, and opens a mechanical switch (approximately 30 sec after the load injector step is executed).

Event Log Error Messages

Cartridge, Flask Load, and Transfer Fluid Sensor Error Messages

41. Fluid Not Detected, No Wet Counts

Symptom: Fluid Not Detected, No Wet Counts.

Dry = 716		Threshold = 1075		Average Wet = 0	
Dry	Wet	Dry	Wet	Dry	Wet
3846	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
Load R1 Cart (lg loop)					

Figure A-12 Sample Event Log Message—Fluid Never Reaches the Sensor

In Figure A-12, the Average Wet value is 0 because no readings were detected above the Threshold. Consequently, there are no Wet values.

<table>
<tr><th>Observation</th><th>Possible Cause</th><th>Recommended Action</th></tr>
<tr><td rowspan="2">Fluid not detected, no wet counts</td><td>Empty bottle.

When a bottle is close to empty, the fluid in the delivery line becomes segmented with argon bubbles, causing delivery to slow until it eventually stops.</td><td>Replace an empty bottle.

Run the respective bottle change procedure so that the delivery line is backflushed before the line is primed. The bottle change procedure is in Chapter 6, “Tests and Procedures.”</td></tr>
<tr><td>Incorrect Set pressure.

<table><tr><th>If...</th><th>Then...</th></tr><tr><td>the Set pressure is too low</td><td>the delivery will slow down and may stop altogether.</td></tr><tr><td>it is too high</td><td>the chemical will be subject to increased outgassing.</td></tr></table></td><td>1. Check the Set and Actual pressures in the Pressures and Temperatures dialog box.

A Set pressure of 0 may be the result of an aborted leak test. Always allow a leak test to finish, or click Next Step to jump to the end of a step.

2. Click Default in the Pressures and Temperatures dialog box to restore the default operating pressures for the system.

3. Turn the heaters back on if necessary. Click Execute. The pressure and vent valves will actuate, causing the Actual bottle pressures to reach the Set pressures. Non-bottle Actual pressures may remain higher than Set pressures until an associated function is activated.

4. Check to see if the Actual bottle pressure follows the Set pressure within ± 0.1 psi after clicking Execute.

There may be a problem with the pressure management system. Contact your local tech support or field service representative for assistance.</td></tr>
</table>

Observation	Possible Cause	Recommended Action
Fluid not detected, no wet counts *(continued)*	Manual regulator pressure too low	Check the pressure by lowering the plumbing plate and looking at the manual regulator pressure gauge. If necessary, set the regulator to 5.5 psi.
	Bottle pressure leak	Remove the leaking bottle and examine the bottle seal. Replace the seal if it is cracked. Run a leak test on the bottle from the Bottle Change dialog box. Make repairs as necessary
	Restricted pressurization path. The delivery pressures are monitored at the pressure management printed circuit board (PCB). A flow restriction between the PCB and a bottle can result in reduced bottle pressure during a delivery.	Run a Leak test on the appropriate bottle, for example the R3 leak test. The actual pressure reported in the event log after the venting portion of the test should be no greater than 0.1 psi. If it exceeds this value, determine the source of the restriction. The restriction will be located between the pressure transducer and the vent valve for the respective bottle. Start with the check valve.
	Restricted fluid delivery path. There is no problem with bottle pressurization.	Determine whether any other deliveries are affected by examining event log and sensor data. Concentrate on flow paths that are common to other affected deliveries. If the problem is unique to a single chemical, check the appropriate delivery line for crimps or restrictions.
	Restricted waste lines	Check the line from the waste bottle to the fume hood. Remove any restrictions such as trapped liquid.

Observation	Possible Cause	Recommended Action
Fluid not detected, no wet counts *(continued)*	Restricted flushing path	Run the procedure, "Testing Three-Way Valves" on page 7-20, in Chapter 7, "Maintenance." to check for restrictions. Remove restrictions if found. Before a load function, the plumbing pathway is flushed with high pressure argon to ensure it is dry. All flushing functions use the high pressure argon supply connected to the respective 3-way valve. The high pressure input to valves 46 and 48 is connected directly to the manual regulator (5.5 psi). For valve 47, it is connected to regulator 8 on the pressure management board.
	3-way valve mechanical failure	Run the procedure, "Testing Three-Way Valves" on page 7-20 in Chapter 7, "Maintenance." to determine whether the valve is switching correctly. If it is not, replace the valve. If a 3-way valve fails to switch from low pressure input to high pressure (5.5 psi) input during a flush function, the effectiveness of the flush will be compromised.

42. Fluid Not Detected, Intermittent Wet Count

Symptom: Fluid Not Detected, Intermittent Wet Count.

Dry = 716		Threshold = 1075		Average Wet = 2744	
Dry	Wet	Dry	Wet	Dry	Wet
2800	180	8	98	78	165
78	183	89	173	78	79
84	187	167	25	74	171
Load R1 Cart (lg loop)					

Figure A-13 Sample event log error message—too many bubbles in liquid slug

When too many bubbles are present, fluid is fragmented as it flows through the sensor. Fragmentation tends to slow down the delivery, resulting in longer than normal initial Dry readings. Because the sensor never detects a sufficient number of consecutive Wet readings:

- The function times out.
- An error is posted in the event log.
- All 18 fields for Dry/Wet readings will contain a non-zero value (Figure A-13).

Observation	Possible Cause	Recommended Action
Fluid not detected, intermittent wet count	Incorrect Set pressure. A low set pressure slows down delivery and may stop it. A high set pressure will increase outgassing to the chemical.	1. Check the Set and Actual pressures in the Pressures and Temperatures dialog box. A set pressure of 0 may be the result of an aborted leak test. Always allow a leak test to finish, or click Next Step to prematurely advance to the end of a step. 2. Click **Default** in the Pressures and Temperatures dialog box to restore the default operating pressures for the system. Turn the heaters back on if necessary. Click **Execute**. The pressure and vent valves will actuate, causing the Actual bottle pressures to reach the Set pressures. Non-bottle Actual pressures may remain higher than Set pressures until an associated function is activated. 3. If the Actual bottle pressure does not follow the Set pressure within ± 0.1 psi after clicking Execute, there is a problem with the pressure management system. 4. Before replacing parts such as the respective pressure transducer, ensure that the tubing connected to the pressure transducer is unrestricted and free of crimps.
	Fume hood vacuum too high. Suction on the vent/waste line is too great.	Gas is pulled out of solution during a delivery, severely segmenting the flow. Ensure that the fume hood vacuum complies with the guidelines listed in the PROCISE/PROCISE *cLC Site Preparation and Safety Manual (P/N 4314377)*.
	Protein sequencer has been sitting idle with the default pressure settings. A significant amount of argon is absorbed by the chemicals. The argon will tend to outgas when the chemical is next delivered.	Adjust all of the bottle Set pressures to 1 psi before leaving the protein sequencer idle.

Injector Sample Loop Fluid Sensor Errors

43. Injection, No Peaks, Sensor Error(s)

Symptom: Injection, No Peaks, Sensor Error(s).

Observation	Possible Cause	Recommended Action
Injection, no peaks, sensor error(s)	Air injection. A small dry reading of 29 (0.15 sec) in the sample loop full sensor error indicates that the injector was actuated almost immediately after the Load Injector function started, and before any liquid could reach the sample loop.	Check the PEEK fittings at ports 5 and 6 of the injector. Installation is correct if the fittings do not leak, and remain in place when you tug on them. See “To reinstall the PEEK fittings:” procedure below.
	The sample loop load sensor was incorrectly initialized	Rerun the Init Sensor procedure.
	Residual liquid was not completely flushed out the injection system prior to the Load Injector step	Check for free flow from the flask through the injector and out to waste. Replace the tubing or fitting if necessary.
	The pick-up line and flask are dirty	Replace the pick-up line and flask if they are dirty. Always replace the pick-up line instead of re-adjusting it to prevent multiple occlusions caused by repositioning the fitting and ferrule.

To reinstall the PEEK fittings:

1. Remove the orange Teflon lines from ports 5 and 6 of the injector.
2. Cut 1 in. off each line. If this makes the lines too short, replace the lines.
3. Route the tubing connecting the injector to the waste bottle through the hole in the panel to the left of the injector.
4. Reconnect the lines to the injector. Finger-tighten the PEEK fittings, then tighten them 1/4-turn more using a wrench.
5. Slide the sensors as close to the PEEK fittings as possible.
6. Gently tighten the pre-tee fittings only enough to hold the sensors in place.

44. Partial Injection, Small Peaks, Sensor Error

Symptom: Partial Injection, Small Peaks, Sensor Error.

Dry = 1019		Threshold = 1530		Average Wet = 3212	
Dry	Wet	Dry	Wet	Dry	Wet
430	1530	10	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0

Figure A-14 Sample event log error message—sample loop full sensor

Observation	Possible Cause	Recommended Action
Partial injection, small peaks, sensor error	Liquid passed the sample loop full sensor prior to the injection.	Reduce the Concentrate Sample step (Function 238) by 5 sec.
		Ensure that two complete loads of S4 were delivered to the flask. To do this, analyze the Flask load loop sensor data file. **Note:** The Flask load loop sensor data file is generated when the Always Report Sensor Data box is checked in the Preferences dialog box of the Sequencer pull-down menu.

45. No Injection, No Peaks, Sensor Error(s)

Symptom: No Injection, No Peaks, Sensor Error(s).

This error will pause the run at the end of the **next** flask cycle. The chromatogram data file from the last cycle will be collected as long as the injection for that cycle was OK. It will appear as the last chromatogram in the SequencePro software file.

The second-to-last chromatogram, therefore, is the data from the flask cycle in which the error occurred. If a chart recorder is being used to collect data simultaneously, it will include data from the cycle in which the error occurred.

Event log data shows that fluid arrived at the sample loop full sensor (sample loop inlet) in the normal time. However, the fluid never made it to the sample loop load sensor (sample loop outlet).

Observation	Possible Cause	Recommended Action
No injection, no peaks, sensor error(s) The tail-end of the slug leaving the flask becomes severely fragmented, slowing delivery down to a halt.	A flow restriction	1. Check the PEEK fittings at ports 5 and 6 of the injector. See "To reinstall the PEEK fittings:" procedure below. Installation is correct if the fittings do not leak, and remain in place when you tug on them. 2. Replace the pick-up line and flask if they are dirty. Always replace the pick-up line instead of re-adjusting it to prevent multiple occlusions caused by repositioning the fitting and ferrule. 3. Reset the protein sequencer. See the procedure below.
	A leaking flask	
	An improperly positioned pick-up tube	

To reinstall the PEEK fittings:

1. Remove the Teflon lines from ports 5 and 6 of the injector.
2. Cut 1/4-in. off each line. If this makes the lines too short, replace the lines.
3. Reconnect the lines to the injector. Finger-tighten the PEEK fittings, then tighten them 1/4-turn more using a wrench.
4. Slide the sensors as close to the PEEK fittings as possible.
5. Gently tighten the pre-tee fittings only enough to hold the sensors in place.

To reset the protein sequencer:

1. Shut down the computer.
2. Power down the protein sequencer.
3. Unplug the MEL card (firmware).
4. Power up the protein sequencer.
5. Power down the protein sequencer.
6. Plug in the MEL card.
7. Power up the protein sequencer.
8. Reboot the computer.

Other Event Log Error Messages

46. Argon Tank Pressure Too Low

Symptom: Argon Tank Pressure Too Low.

Argon tank pressure is too low. The sequencer is paused.

Figure A-15 Event log message—argon tank pressure too low

Observation	Possible Cause	Recommended Action
Argon tank pressure too low	The argon supply pressure drops below 60 psi to generate message in Figure A-15	Readjust the argon tank regulator to 70 to 80 psi.
		Check the 1/4-in. tubing to see if it is securely connected to the high-pressure transducer on the pressure-control board (visible from the top of the instrument after removing the top cover). Secure the tubing.

47. Cannot Reach Set Temperature

Symptom: Cannot Reach Set Temperature.

During the Begin step of a cycle, the cartridge, flask and column temperatures are monitored, and an error message is generated if any fail to reach set point within 20 min.

Unable to reach cartridge, flask or column temperature setpoints. The sequencer is paused.

Figure A-16 Event log message—unable to reach temperature setpoints

Observation	Possible Cause	Recommended Action
Cannot reach set temperature	Temperature difference between ambient and target is too great to cover in 20 min	Test the heater(s) from the Pressures and Temperatures View. Enter a target temperature and click on Execute.
	Thermal fuse on the heater printed circuit board is bad	Contact your local tech support or field service representative for assistance.

48. Communication With HPLC Lost

Symptom: Communication With HPLC Lost.

The protein sequencer can no longer communicate with the 140 pump via the RS232 cable.

Communication with the HPLC was lost. Reset the HPLC.

Figure A-17 Event log message—communication with HPLC was lost

Observation	Possible Cause	Recommended Action
Communication with HPLC lost	The 140 pump is not turned on	Turn on the pump.
	The RS232 cable between the 140 pump and the protein sequencer is not properly seated	Reseat the RS232 cable. Restart communication with the 140 pump. Cycle the pump power by turning the instrument off and on.

49. Event Buffer Overrun

Symptom: Event Buffer Overrun.

The message shown in Figure A-18 indicates communication between the computer and the protein sequencer or the protein sequencer and the HPLC pump was lost, and the protein sequencer posted errors in the event buffer.

Because this error is normally the result of a communication failure, this message will not be transferred from the protein sequencer to the computer until communication is re-established.

Event buffer overrun. Some event messages may have been lost.

Figure A-18 Event log message—event buffer overrun

Observation	Possible Cause	Recommended Action
Event buffer overrun	A protein sequencer–computer or protein sequencer–HPLC pump communication failure	Verify that: • All communication cables are correctly attached. • Procise software shows that the HPLC pump is attached. If there is a problem, then: 1. Quit the software. 2. Power off the compute, pump and protein sequencer. 3. Reseat all cables. 4. Power on the pump, protein sequencer and computer. 5. Launch the PROCISE software.

50. Injector Position Error, Sensor Errors

Symptom: Injector Position Error, Sensor Errors.

When the “load injector” function is started, the Rheodyne valve must be in the load position. When starting step (a) of cycle (b), the Rheodyne valve was in the inject position.

Figure A-19 Event log message—start injector position error

When the “Load injector” function is finished, the Rheodyne valve must be in the inject position. When finishing step (a) of cycle (b), the Rheodyne valve was in the load position.

Figure A-20 Event log message—Finish Injector Position Error

Observation	Possible Cause	Recommended Action
Injector position error, sensor errors	The injector did not move to the correct position. Either of the event log messages shown in the figures above is generated.	Follow the troubleshooting information provided for “45. No Injection, No Peaks, Sensor Error(s)” on page A-38.
	The flask cycle does not include a Load Position step prior to the Load Injector step.	Modify the function to include a Load Position step prior to the Load Injector step. Instructions for modifying cycles are on page 5-5 in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients.”
	The valve switching and the vacuum out-gassing mechanism is not working properly.	Check the injector actuator mechanism by moving the injector between the load and inject positions while in Manual mode.

51. Insufficient Memory

Symptom: Insufficient memory.

Insufficient data collection memory for cycle (a). The sequencer is paused.

Figure A-21 Event log message—insufficient data collection memory for cycle

Observation	Possible Cause	Recommended Action
Insufficient memory **Note:** This error is typically the result of a protein sequencer-computer communication failure. As such, the message is not transferred from the protein sequencer to the computer until communication is re-established.	Loss of communication with the computer or the computer's hard drive is full. During the Begin step of a cycle, the system control software determines whether there is enough free space is available in the data buffer to collect the chromatogram for that cycle. If the amount of memory is insufficient, the protein sequencer will wait at the Begin step for up to 4 min. If enough memory is still not available, the message shown in Figure A-21 is sent to the event log.	Verify that there is sufficient space on the computer's hard drive to collect and store new data. At least 100 MB of free space should be available to avoid problems. If the hard drive is full, delete unnecessary files.

52. Invalid Sensor Dry Reading

Symptom: Invalid Dry Reading.

Sensor (a) does not have a valid dry reading.

Figure A-22 Event log message—invalid sensor dry reading

Observation	Possible Cause	Recommended Action
Invalid sensor dry reading	Init Sensor procedure not run after resetting the memory card.	Run the Init sensor procedure to re-establish Dry readings. The procedure is in Chapter 6, “Tests and Procedures.”

Overview:

During the Init Sensor procedure, the dry reading for each fluid sensor is automatically established at the start of each sequencing run. These readings are stored in memory until overwritten during execution of a subsequent Init Sensor procedure.

Dry readings are lost when the instrument is reset by pulling the memory card. If a function utilizing a sensor is executed:

- After a reset
- Prior to running the Init Sensor procedure

The message shown in Figure A-22 is sent to the event log.

53. Power Failure

Symptom: Power Failure.

Power failure occurred on mm/dd/yy, at hh:mm:ssc

Figure A-23 Event log message—power failure

A power fail occurred while sequencing. The run will be paused on the End step of the active cycle.

Figure A-24 Event log message power failure during a run

Observation	Possible Cause	Recommended Action
Power failure	A power failure occurred	If no other instruments in the lab experienced the same power failure, make sure all power cords for the system are properly connected to the instruments and power outlets.

54. Vacuum Assist Activating

Symptom: Vacuum Assist Activating.

Note: Periodic activation of one time/day is normal.

Vacuum assist activated.

Figure A-25 Event log message—vacuum assist activated too often

Observation	Possible Cause	Recommended Action
Vacuum assist activating	The vacuum system is leaking	Contact your local tech support or field service representative for assistance.
	Bad Clippard valve	

Leak Test Error Messages

55. Actual Pressure > 5.5 psi

Symptom: Actual Pressure > 5.5 psi.

Observation	Possible Cause	Recommended Action
Actual Pressure > 5.5 psi	The Actual pressure for a particular regulator in the Pressures and Temperatures dialog box is above 5.5 psi (the manual regulator setting)	Check to see if the manual regulator gauge is reading 5.5 psi. If it is not, adjust the gauge while executing Function 137, Flush Input Block. Reset the protein sequencer. See “To reset the protein sequencer:” procedure below.
	The pressure transducer is bad	Replace the pressure transducer for the affected position. The replacement procedure is in Chapter 7, “Maintenance.” If the problem persists, the pressure control board or the I/O board may be malfunctioning. Contact your local tech support or field service representative for assistance.

To reset the protein sequencer:

1. Shut down the computer.
2. Power down the protein sequencer.
3. Unplug the MEL card (firmware).
4. Power up the protein sequencer.
5. Power down the protein sequencer.
6. Plug in the MEL card.

7. Power up the protein sequencer.
8. Reboot the computer.

56. Leak Tests Fail

Symptom: Leak Tests Fail.

Observation	Possible Cause	Recommended Action
Leak tests fail	An incorrect grade of argon is being used	Replace it with the proper grade of argon (99.998% purity or greater).
	The vent line is obstructed	Look for obstructions such as condensation in the vent line (the vent line runs from the protein sequencer to the fume hood). Remove the obstruction.
	Manual regulator not set to 5.5 psi	Adjust the regulator setting to 5.5 psi.

57. Vent Test Fails

Symptom: Vent Test Fails.

Observation	Possible Cause	Recommended Action
Vent test fails	A check valve malfunction	Replace the malfunctioning check valve (the one connected to the bottle failing the vent test).
	A vent line obstruction	Clear obstructions from the vent line.
	Malfunctioning Angar vent valve	Replace or repair the Angar valve.
	Blockage between the pressure transducer and the waste bottle	Find and remove the blockage between the pressure transducer and the waste bottle.

58. Flask Vent Test Fails

Symptom: Flask Vent Test Fails.

Observation	Possible Cause	Recommended Action
Flask vent test fails	The X3 pressure check valve is malfunctioning	Replace the X3 pressure check valve.

59. Leak Test Fails, Pressure High

Symptom: Leak Test Fails, Pressure High.

Observation	Possible Cause	Recommended Action
Leak test fails, pressure high	A Lee valve on the pressure control board is leaking	Call Applied Biosystems for service.

60. Cartridge Leak Test Fails

Symptom: Cartridge Leak Test Fails.

Observation	Possible Cause	Recommended Action
Cartridge leak test fails	If all cartridges fail the leak test, there is a leak somewhere before the cartridge	Run the cartridge reagent block and cartridge input block leak tests listed in Chapter 6, “Tests and Procedures.”
	The seal and/or filter in the cartridge is worn	Replace the seal and filter in the reaction cartridge. Be sure to centrally position the filter.
	A cartridge ferrule is scratched	1. Examine the ferrule for scratches. 2. If it is a multi-cartridge instrument, swap out cartridge components until the scratched ferrule is identified.
	The cartridge is dirty	1. Clean dirty reaction cartridges. 2. Remove and clean the glass cartridge blocks. Instructions are in Chapter 7, “Maintenance.” 3. Sonicate the cartridge and cap in methanol to clean the threads.

Miscellaneous Problems

Communication Errors

61. Communication Lost Between Computer and Protein Sequencer

Symptom: Communication Lost Between Computer and Protein Sequencer.

Under normal circumstances, the COM light inside the protein sequencer is on, indicating the computer and protein sequencer are communicating. This light is visible through the front panel visor of the protein sequencer.

If the light turns off, communication has been lost. Consequently, sequence data and event information cannot be loaded from the protein sequencer to the computer. If this occurs, the run will be paused to protect your data.

Observation	Possible Cause	Recommended Action
Communication lost between computer and protein sequencer	A dialog box on the computer generated by the PROCISE application will cause the COMM light to turn off.	Select one of the prompts in the dialog box to re-establish communication.
	The computer has locked-up.	Restart the computer.
	The communication cable between the computer and protein sequencer is loose.	Turn off the power to the two instruments, and reseat the cable.
	The protein sequencer has locked up.	Reset the protein sequencer. See “To reset the protein sequencer:” below.

To reset the protein sequencer:

1. Shut down the computer.
2. Power down the protein sequencer.
3. Unplug the MEL card (firmware).
4. Power up the protein sequencer.
5. Power down the protein sequencer.
6. Plug in the MEL card.
7. Power up the protein sequencer.
8. Reboot the computer.

UV Detector Errors

62. Beeping UV Detector

Symptom: The UV Detector Is Beeping.

Observation	Possible Cause	Recommended Action
Beeping UV detector	The flowcell is leaking	1. Stop the 140 pump. 2. Soak up the spill. 3. Rebuild the flowcell. Follow the procedure in Appendix 7, “Maintenance,” to rebuild the flowcell.
	The sensor in the detector is not initialized correctly	Cycle the power on the detector.
	The sensor in the detector is not positioned correctly	Reposition the sensor so it is not touching the drip tray. Cycle the power on the detector.

Chemistry Overview

B

This appendix contains the amino acid table, and overviews of the N-terminal and C-terminal sequencing chemistries.

The following topics are contained in this appendix:

Amino Acid Table

Abbreviations Table

Amino Acid	Abbreviation	Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Amino Acids Modified – C-Terminal Sequencing

Arg Most of the Arg is acetylated during the initial C-terminal activation. This derivative is identifiable by a unique retention time, but has not been characterized and is not included in the ATH-AA standard.

Asp and Glu Both of these amino acids with side-chain carboxylic acid groups are modified to piperidine amides during the automated chemistry. This prevents the side-chain carboxyls from interfering in the sequencing chemistry and provides easily detectable derivatives for these amino acids. The piperidine amide derivatives of Asp and Glu are included in the ATH-AA standard.

Cys Cys residues must be reduced and alkylated before sequencing for unambiguous identification. Acrylamide alkylation provides a Cys derivative with good stability to the sequencing chemistry and good chromatographic positioning. Without prior modification, Cys residues are detected as dehydroalanine, and as such are indistinguishable from Ser residues. The acrylamide derivative of Cys is included in the ATH-AA standard.

Gly (at the C-terminus) Gly, at the C-terminus of the protein, is detected in unacetylated and acetylated forms. Internal Gly residues are detected only in unacetylated form. Acetylated Gly is not included in the ATH-AA standard.

His Some His is recovered in unmodified form during sequencing. However some His is acetylated during the initial C-terminal activation or modified in other ways. These derivatives are not characterized.

Lys The standard protocol is to manually pretreat samples with phenylisocyanate which yields e-phenylcarbamyl-Lys. This Lys derivative is included in the ATH-AA standard.

Ser and Thr During the initial activation, Ser and Thr are acetylated and subsequently undergo ß-elimination, yielding dehydroalanine from Ser and the corresponding dehydro-analog from Thr. The ATH standards of Ser and Thr also undergo ß-elimination during preparation for chromatography and are detected as the dehydro-analogs.

Tyr Tyr is detected in unacetylated and acetylated forms. Both Tyr and acetylated-Tyr are included in the ATH-AA standard.

Expected C-Terminal Sequencing Results

Yields

A key point to be realized is that Pro residues stop sequencing. Callable sequence stops at the residue before Pro. In general, as opposed to N-terminal sequencing, C-terminal sequencing performance is sequence dependent. Initial yield and sequencing efficiency vary according to the specific amino acid sequence being analyzed.

Typical Initial Yields	If...
15-30%	Ala, Asn, Gln, Gly, Ile, Leu, Lys, Met, Phe or Val is at the C-terminus.
5-15%	Arg, Cys, Glu, His, Thr or Tyr is at the C-terminus.
<5%	Asp or Ser is at the C-terminus.

What is the effect of either Asp or Glu at the initial C-terminus of the protein?

Asp and Glu, during initial activation, can also form the respective symmetric anhydrides between the side-chain and alpha-carboxylic acid groups. This side-reaction contributes to a significant decrease in the initial yields, particularly for Asp.

What are the effects of specific residues on the efficiency of C-terminal sequencing?

Residues that...	Are...
do not reduce sequencing efficiency	Ala, Asn, Cys, Gln, Gly, Ile, Leu, Lys, Met, Phe, Tyr, Trp and Val.
may slightly reduce sequencing efficiency	Arg, Glu, His and Thr
may reduce sequencing efficiency	Asp and Ser
may be impossible to unambiguously identify and may reduce the sequencing yield below callable levels	consecutive Ser and/or Thr

Typical Apomyoglobin Cycle 1 Results

The chromatogram examples of Apomyoglobin cycle 1 (Glycine) below are at 50 mAUFS and taken from two different sequencers. They show Glycine residue from 1 nmol of horse apomyoglobin applied to a ProSorb® filter. The methylimidazole catalyzed acetylation of Ser and Thr hydroxyls also causes acetylation of Gly (G') at the C-terminus.

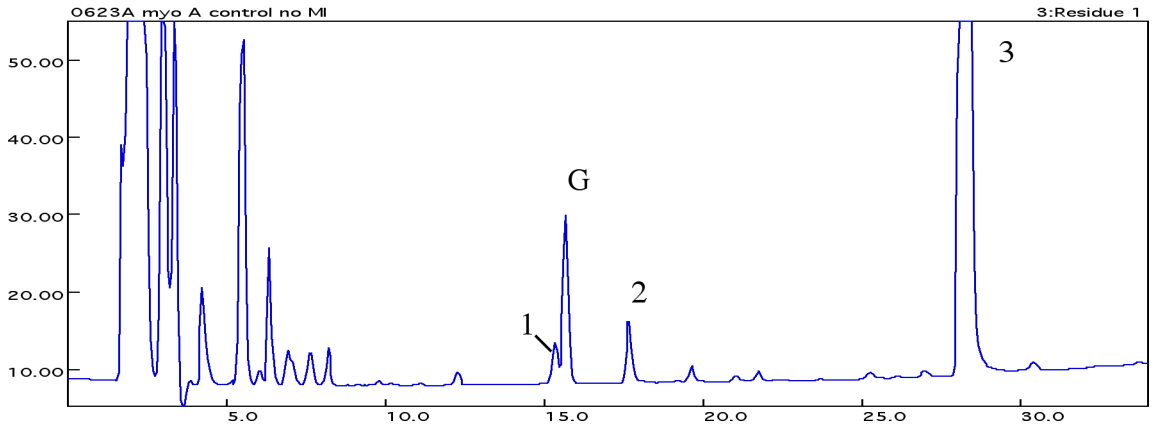


Figure B-1 Cycle 1: Apomyoglobin (1nmol), no OH capping

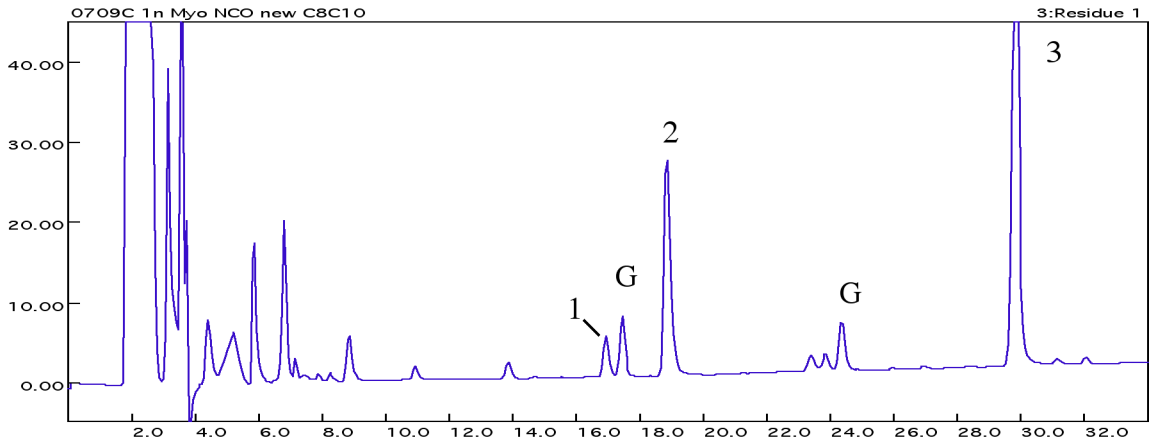


Figure B-2 Cycle 1: apomyoglobin (1 nmol), with OH capping

Typical Results for Apomyoglobin Yield

Yield	%
Initial	15 - 30
Repetitive	65 - 80%

Major Chemistry Artifact Peaks

Peak #	Location	Chemical
1	Peak before Gly at 5 - 15 mAU	not chemically identified
2	Peak before Ala at 5 - 40 mAU	not chemically identified
3	Peak at 80 - 240 mAU	Naphthylmethylthiocyanate, a reaction product of bromomethylnaphthalene and tetrabutylammonium thiocyanate

Optimizing N-Terminal Sequencing Chemistry

N-Terminal Sequencing and PITC Reaction

N-Terminal Sequencing Overview

The goal of performing N-terminal sequencing on an unknown protein/peptide sample is to unambiguously identify as many amino acids as possible using the least amount of sample.

The length of the protein sequence that can be determined is limited by the chemical efficiency of the Edman degradation. Length is also limited by these sample attributes:

- The purity of the sample
- Amount of the sample
- Molecular weight of the sample
- Conformation of the sample

Because the chemical efficiency is less than 100%, the amount of sample you can sequence decreases slightly with each successive degradation cycle.

Reaction of PITC

With the exception of the initial coupling, the reaction of phenylisothiocyanate (PITC) with the amino terminus or termini proceeds nearly quantitatively. The particular amino acid undergoing reaction, or the local structure of the peptide chain, has little effect on the efficiency of the coupling reaction.

The Coupling Reaction

Coupling Reaction Overview

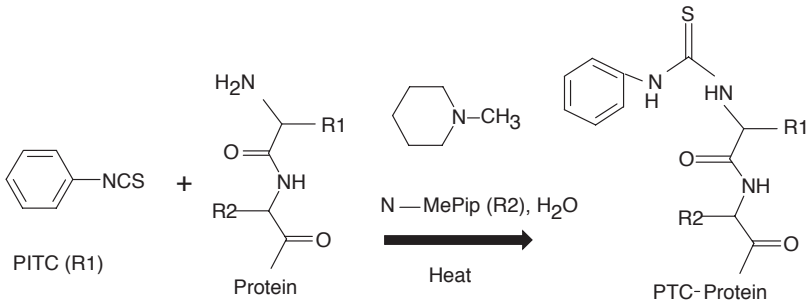


Figure B-3 Coupling reaction

Coupling occurs when the free amino terminus of a protein or peptide reacts with PITC to create a phenylthiocarbamyl (PTC) protein or peptide. The coupling reaction has two main parts:

- Delivery of PITC and base vapor to provide the basic environment necessary for coupling.
- Drying and washing to remove excess reagent and reaction by-products.

Coupling and PITC

The coupling reaction used for samples bound to PVDF membrane differs slightly from the coupling for samples applied to glass fiber. Sequencing cycles have typically been written for samples applied to a hydrophilic support. The hydrophilicity of the support facilitates the absorption of a small amount of water which is necessary for the efficient coupling of PITC to the amino terminus of the sample.

Coupling and PVDF

PVDF membrane is routinely used for electroblotting samples from gels. It can also be used to remove excess salt and buffers from samples prior to sequencing. The membrane binds proteins through hydrophobic interaction. Because PVDF membrane is hydrophobic, it tends to repel rather than absorb water.

Coupling Base Delivery

The first step of coupling in all chemistry cycles is the delivery of R2 vapor to the cartridge. This raises the pH of the sample, and deprotonates the free amino groups for reaction with PITC. The length of this delivery should be at least 20 to 30 sec, but it can be increased to as much as 120 sec without negative impact. The length of the base deliveries after the PITC delivery should be at least 120 sec. To minimize the modification of aspartic and glutamic acid residues, avoid making the total cumulative base delivery time longer than 700 sec.

Under the basic conditions necessary for coupling, aspartic and glutamic acid residues are slowly modified by the reaction of the side chain carboxylic acid group with aniline. The derivative of aspartic acid can be found just before the DPTU peak in the chromatogram; the derivative of glutamic acid is just after DPTU. The extent of modification of aspartic acid and glutamic acid residues increases slightly with each sequencing cycle. The effect is more pronounced for glutamic acid residues. The rate of modification of aspartic and glutamic acid residues also increases with the coupling temperature and is more noticeable with increasing amounts of sample.

PITC Delivery

The standard chemistry cycles provided with this system include three deliveries of PITC during the coupling reaction. However, customized cycles can specify more or less than three deliveries. If two PITC deliveries are used, increase the base deliveries to 270 to 300 sec each. More than three PITC deliveries might be required to sequence very large amounts of sample, or to sequence a sample on multiple pieces of PVDF, when contact of reagent and membrane is a concern.

A short argon delivery occurs after each PITC delivery to evaporate heptane. Residual heptane would interfere with the coupling reaction by keeping most of the PITC in the organic phase. The drying time should be at least 20 sec to ensure adequate removal of heptane. Remember, a base delivery should always precede the first PITC delivery to the cartridge.

Coupling Temperature

The temperature of the cartridge during coupling is set high enough to promote fast, efficient reaction of PITC with the amino-terminal amino group without excessive side-reactions. For example, the standard pulsed-liquid and pulsed-liquid blot cycles use a coupling temperature of 48 °C. Under the basic conditions necessary for coupling, aspartic and glutamic acid residues are slowly modified by reaction of the side chain carboxylic acid group with aniline.

The rate of modification of aspartic and glutamic acid residues is slightly higher on glass fiber than on PVDF. The lower coupling temperature for glass fiber provides a rate comparable to PVDF at the higher temperature.

Drying After Coupling

Drying after coupling eliminates the water absorbed by the polybrene during the coupling reaction. Some of the reaction chemicals will also be reduced during this step, but the subsequent wash will remove the bulk of the chemistry by-products. The drying time can be extended without the loss of residues. The goal is to eliminate as much water as possible before the wash and cleavage steps. This will prevent sample washout, and hydrolysis of the peptide chain during the cleavage.

Postcoupling Wash

The postcoupling wash removes as much of the coupling reagents and reagent by-products as possible before cleavage. A combination of solvents S2B and S3 are used. The washing scheme of short solvent deliveries alternated with brief cartridge wait steps reduces the likelihood of sample washout. This scheme also results in maximum wash efficiency with minimal solvent consumption.

The first delivery of solvent to the cartridge is S3, the less polar solvent. S3 solvent reduces the possibility of sample washout from the reaction cartridge. Increasing the volume of solvent used for this wash will reduce the chemistry background but may increase sample loss from the cartridge due to washout, particularly if short hydrophobic peptides are being sequenced. In particular, lengthy S2B washings will aggravate sample washout.

Drying after the postcoupling wash requires no special considerations other than completely drying the sample to prevent washout. Typically there is no danger of overdrying the sample at this point.

The Cleavage Reaction

Cleavage Reaction Overview

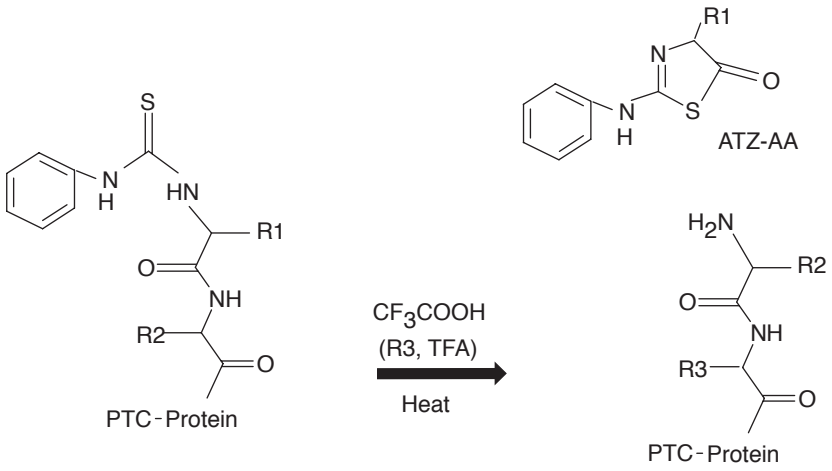


Figure B-4 Cleavage reaction

Producing a cleavage reaction requires the use of a strong acid. A balance must be struck between complete cleavage of the ATZ-amino acid, and unwanted acid cleavage at other sites along the peptide chain; cleavage efficiency varies according to the amino acid derivative being cleaved as well as the next amino acid in the chain.

Incomplete Cleavage

Incomplete cleavage of the ATZ-amino acid is referred to as *lag*. The remaining, uncleaved portion of the current N-terminal amino acid appears in the chromatogram in the following cycle with the next amino acid.

Lag increases with each cycle in a sequencing run. Depending on the particular amino acids in the sequence, lag can be the primary reason a sample stops producing useful sequence data.

Nonspecific Cleavage

Repetitive exposure of the sample to strong acid can result in cleavage between amino acids elsewhere in the peptide chain. Each time nonspecific cleavage of the peptide chain occurs, a new N-terminus is generated which can react with PITC. This will increase the *amino acid background*—the presence of other PTH-amino acids in the chromatogram which do not reflect the true N-terminal sequence.

Amino Acid Background

At the start of a sequencing run, the amino acid background from nonspecific cleavage is low. Background increases with each sequencing cycle. Fortunately, nonspecific cleavage is sequence specific, so only peptide bonds between amino acids will be cleaved. This keeps the amino acid background rate from cycle to cycle quite low.

For proteins with labile amino acid sequences and very large proteins, amino acid background increases more rapidly. In practical terms, ≤10 pmol of a 100 to 200 amino acid protein may provide 40 to 50 cycles of interpretable sequence, while the same amount of a 2000 amino acid protein will typically provide only 10 to 15 cycles of sequence.

Cleavage, the TFA-catalyzed process

Cleavage, whether pulsed-liquid or gas-phase, is the trifluoroacetic acid (TFA)-catalyzed process of removing the PTC-amino acid from the N-terminal end of the sample. Under strong acidic conditions, the peptide chain is cleaved at the peptide bond nearest the PTC-amino acid derivative, resulting in the release of an ATZ-amino acid. Because cleavage is not a hydrolytic process, the sample should ideally be as free of water as possible to minimize nonspecific hydrolytic cleavage of the peptide chain.

Pulsed-Liquid Cleavage

Pulsed-liquid cleavage is performed by system delivery of a small aliquot of TFA to the cartridge on a stream of argon. The system seals off the reaction chamber to allow the cleavage to take place. Pulsed-liquid cleavage proceeds faster than gas-phase cleavage. The standard pulsed-liquid cleavage time is 300 sec at 48 °C.

Certain samples may benefit by varying the cleavage conditions. For example, very large protein samples may sequence better using a shorter cleavage time to minimize amino acid background generated from nonspecific cleavage of certain peptide bonds. Cleavage of the peptide bond after certain amino acids, particularly proline, proceeds more slowly than after other amino acids and will benefit from an extended cleavage time or increased temperature.

You can extend cleavage for proline residues up to 600 sec, twice as long as a standard cleavage. Alternatively, you can increase the temperature of the cleavage to 55 °C. These extreme cleavage conditions should be used only when needed, since the rate of sample degradation significantly increases when they are used for every cycle.

Gas-Phase Cleavage

Gas-phase cleavage is performed by delivering TFA vapor through the active cartridge for a prescribed period of time. Gas-phase cleavage requires more time than pulsed-liquid phase cleavage. As a result, the standard gas-phase cycles are approximately 600 sec—5 min longer than pulsed-liquid cycles. For optimum results, the R3 pressure setting can be reduced to 0.3 to 1.0 psi.

Too high a TFA flow rate through the cartridge will result in higher than expected lag. Reduce the R3 regulator pressure (if the lag per cycle for gas-phase cleavage is higher than for pulsed-liquid). Gas-phase cleavage cycles tend to be somewhat cleaner than pulsed-liquid cycles; the level of chemistry artifact peaks is usually slightly lower. Gas-phase cleavage may also help reduce washout of hydrophobic peptides.

Drying After Cleavage

Drying times after cleavage must strike a balance between the recovery of particular residues and excessive washout if the sample is still too acidic when extractions are done. Avoid overdrying samples after cleavage, since overdrying will drastically reduce recovery of basic residues. Overdrying can also result in the poor extraction of charged residues, and dehydration of labile residues.

A 40-sec drying time is used in the standard pulsed-liquid cycles. Incomplete drying may result in lowered repetitive yields due to sample washout. If sample washout is of greater concern than the recovery of positively charged residues, extend the drying time after cleavage.

ATZ Extraction and Transfer

Overview

After the system completes cleavage and the sample is dried, the ATZ-amino acid is extracted from the cartridge and transferred to the flask. The best method for extracting ATZ differs slightly for the various sample types. Coupling of the new amino terminus can begin once the transfer is complete.

Liquid Samples

Samples applied to glass-fiber disks with polybrene are extracted the same way, whether sequenced using gas or liquid cleavage. Each glass–fiber cycle has two ATZ extractions. The first extraction is done with S3 (butyl chloride); the second with S2B (ethyl acetate). For each extraction, solvent is delivered to the cartridge outlet sensor, is allowed to incubate with the sample for 10 sec, and is then transferred to the flask with argon.

S2B, which is more polar than S3, improves the recovery of polar residues, particularly histidine, arginine, aspartic acid and glutamic acid. Using S3 for the first extraction reduces the possibility of polybrene/sample washout. The argon delivery after each extraction must be long enough to transfer the contents of the cartridge to the flask, and dry the cartridge outlet sensor. If droplets of liquid remain at the outlet sensor, incomplete transfer occurs and low deliveries result.

Flask Chemistry

Once cleaved, the ATZ-amino acid is extracted from the cartridge and transferred to the flask for conversion into the more stable PTH-amino acid derivative. In preparation for the transfer, a small volume of 10% acetonitrile (S4C) is delivered to the flask. The presence of S4C reduces the modification of certain amino acid residues, particularly serine and threonine.

Pre-Conversion Drying

During and immediately following the transfer, the liquid in the flask is bubbled to evaporate the S3 and S2B transferred from the cartridge. Sample volume is also reduced to 10 to 20 µL. At this point in the conversion cycle, the sample should never be completely dried. Completely drying the sample before conversion reduces the recovery of labile residues, particularly serine and threonine.

Conversion

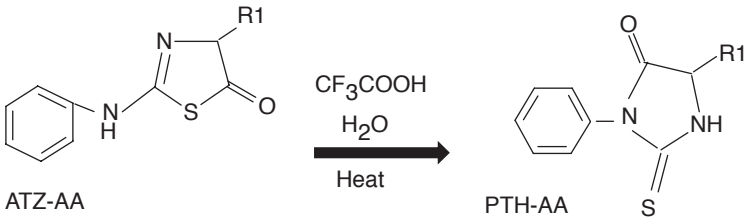


Figure B-5 Conversion reaction

Conversion of the ATZ-amino acid into a PTH-amino acid takes place in an aqueous acid medium. A small loop load of R4 is added to the flask and allowed to incubate with the sample for approximately 10 min. A small load of R4 is used instead of a large load to reduce the drying time required after conversion.

Post-Conversion Drying

After conversion, the sample must be completely dried to remove all the TFA. TFA interferes in the chromatography of early eluting PTH-amino acids. In the standard cLC flask cycles, the flask appears to be dry 180 to 200 sec before the end of the standard flask dry step that follows the Post-conversion Dry step. This additional drying time will not adversely affect the recovery of the PTH-amino acids. PTH-Amino Acid Solubility

The dried PTH-amino acid in the flask is dissolved in 10% acetonitrile (S4C) for subsequent transfer to the injector loop. Two large loop loads of S4C are used to dissolve the sample in the standard flask cycles. Bubbling the contents helps dissolve the sample.

Sample Transfer and Injection

Once the sample has been reconstituted in the flask, it is transferred to the HPLC injector loop. Transfer is accomplished by pressurizing the flask with argon, and driving the sample out through the pickup line into the injector loop. When the Sample Loop Load sensor detects fluid, the injector valve is switched from the load to the inject position, moving the sample into the HPLC solvent stream. The gradient program and data collection begin.

Table B-1 Steps (functions) in flask cycles

Step	Description
Function 227, Prepare Pump	Downloads the gradient program to the 140 pump from the PROCISE cLC control software. After the download is complete (30 to 60 sec), the 140 pump will start, pressurize and run at the initial gradient conditions.
Function 226, Load Position	Sets the injection valve in the load position. Must precede the Load Injector step for the sample loop to be flushed before sample transfer to the sample loop.

Table B-1 Steps (functions) in flask cycles *(continued)*

Step	Description
Function 221, Flush Injector	Flushes the sample loop from valve 44. Does not flush through the flask. Must precede the Load Injector step.
Function 225, Load Injector	Activates the sample loop sensors. Transfers sample from the flask to the HPLC sample loop.

Sample Volume

The volume of sample transferred to the injector loop is determined by the size of the loop loads sent to the flask. The standard volume of a large loop load of S4C is 60 µL. Two loads to the flask provide a total sample volume of 120 µL.

Bubbling in the flask reduces the amount of acetonitrile in the sample, reduces the sample volume, and insures proper binding of the PTH-amino acids to the column.

Injection Percentage

The standard injection percentage for the PROCISE cLC protein sequencer is 55–65%. This percentage was selected to provide consistent fluid detection at the Sample Loop Load and Sample Loop Full sensors.

A procedure for optimizing the injector percentage is listed on page 4-2. Optimize the injector percentage if a large number of Sample Loop Full error messages begin to appear in the event log.

PROCISE C Chemistry Overview

Applied Biosystems Sequencing Method

C-Terminal Sequencing

Obtaining sequencing information from a protein by chemical methods, either from the carboxyl (C)-terminus or the amino (N)-terminus, requires stepwise derivatization, specific cleavage of the derivatized amino acid residue, and accurate identification of the cleaved amino acid. During Edman sequencing, the N-terminus is readily derivatized with phenylisothiocyanate (PITC), and the cleavage step is facilitated by an intramolecular cyclization. The carboxyl end of a protein is more difficult to react selectively and efficiently, and the cleavage of a derivative formed at the C-terminus of a protein requires breaking a stable amide bond which is not significantly different from the other amide bonds in a protein or peptide.

The traditional approach for obtaining sequence information from the C-terminus has centered on the activation of the carboxyl group, derivatization to a thiohydantoin, and hydrolysis of the thiohydantoin from the peptide which regenerates a free carboxyl moiety. The thiohydantoin approach is attractive because of the similarity of the thiohydantoin to the phenylthiohydantoin (PTH) derivative formed in Edman sequencing.

Benefits to the System

The benefits of the Applied Biosystems sequencing method are:

- Selective cleavage of the ATH-amino acid with simultaneous derivatization of the C-terminus into a thiohydantoin.
- The introduction of a tag onto the cleaved amino acid derivative.
- Elimination of the need to return to a free carboxylic acid at the C-terminus.

Overview of the Sequencing Method

The chemical steps involved in the patented Applied Biosystems sequencing method are presented in Figure B-6. A proteinyl-thiohydantoin is formed at the C-terminus to initiate sequencing. The unique feature of the Applied Biosystems method is an aryl-alkylation of the thiohydantoin. Alkylation of the thiohydantoin ring results in a C-terminal derivative that is readily cleaved from the parent protein. Using thiocyanate anion ($\{NCS\}^-$) under acidic conditions, the alkylated thiohydantoin (ATH) amino acid is cleaved, and a new thiohydantoin is formed at the C-terminus.

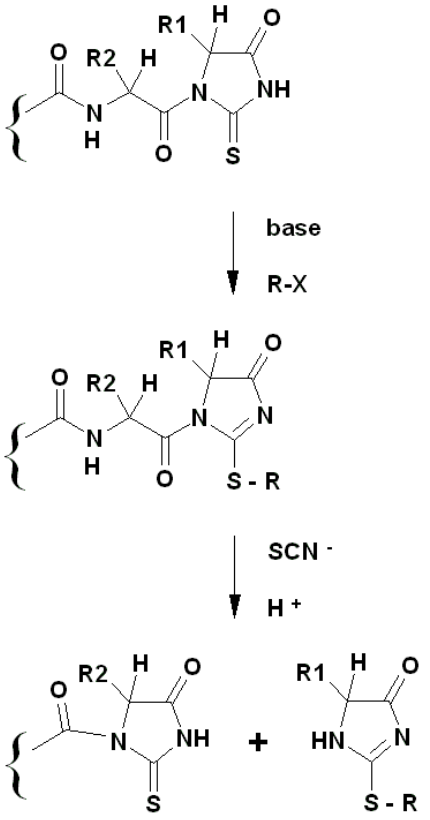


Figure B-6 The Applied Biosystems alkylation chemistry for automated C-terminal protein sequence analysis

Activation of the C-Terminal Carboxylic Acid

As illustrated in Figure B-7, formation of the first thiohydantoin at the C-terminus requires a unique cycle for activation of the free carboxylic acid group, followed by derivatization with {NCS}–. A mixture of acetic anhydride and lutidine is used for the activation of the carboxyl groups. Under basic conditions, an oxazolone forms exclusively at the C-terminus. The oxazolone reacts with tetrabutylammonium isothiocyanate to form a C-terminal thiohydantoin under conditions made acidic with TFA vapor.

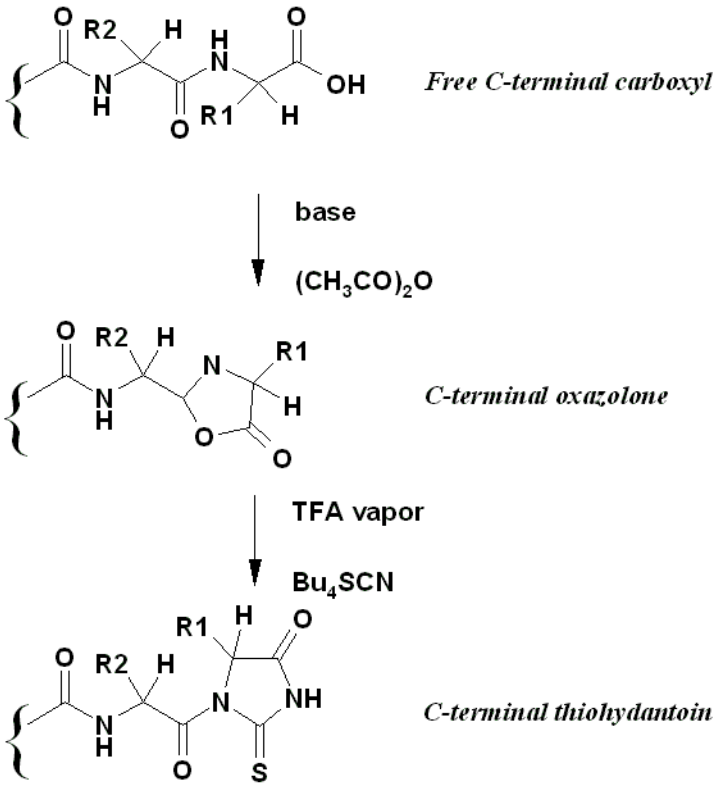


Figure B-7 Activation of C-terminal carboxylic acid to form a thiohydantoin

Converting Amino Acids into Derivatives

Conversion Table

Since the introduction of the alkylation chemistry, various treatments have been explored for modifying reactive side-chains. Special cycles have been developed to convert amino acids with reactive side-chains into derivatives that can be more readily sequenced through and detected chromatographically. Table B-2 lists the amino acid residues, the nature of the modification to enhance sequencing (if required), and the strength of the signal seen during HPLC analysis.

Note: For sequencing, the side-chain carboxyl groups of the aspartic (Asp) and glutamic (Glu) acid are derivatized into piperidine amides. The hydroxyl groups of serine (Ser) and threonine (Thr) must be *capped* (acylated) to sequence through them.

Table B-2 Anticipated C-terminal sequencing results

AA Residue	Pretreatment Modification	Signal Intensity	ATH Derivatives	Peak Identification	Additional Comments
Ala	None	Strong	1	Ala-ATH	—
Arg	None	Medium to low	2	Arg-ATH and acetylated/ alkylated Arg-ATH	The identify of the modified arginine is not known. It elutes before Tyr-ATH.
Asn	None	Strong	1	Asn-ATH	—
Asp	Piperidine amidation	Medium	1	Asp-pip amide-ATH	May react with PIC to form small amount of anilide.
Gln	None	Strong	1	Gln-ATH	—
Glu	Piperidine amidation	Medium	1	Glu-pip amide-ATH	May react with PIC to form a small amount of anilide.
Gly	None	Strong	1	Gly-ATH	C-terminal Gly is split due to acetylation.
His	None	Medium to low	1	His-ATH	May be acetylated or alkylated.
Ile	None	Strong	1	Ile-ATH	Diastereomers not resolved.
Leu	None	Strong	1	Leu-ATH	—
Lys	PIC derivatization of epsilon amine group	Strong	1	PIC-Lys-ATH	If not PIC treated, acetylated Lys is seen, which coelutes with Gly-ATH.
Met	None	Strong	1	Met-ATH	—
Phe	None	Strong	1	Phe-ATH	—
Trp	None	Medium	1	Trp-ATH	—
Tyr	None	Medium	2	Tyr-ATH and acetylated Tyr-ATH	PIC pretreatment reduces the amount of acetylated product.

Table B-2 Anticipated C-terminal sequencing results *(continued)*

AA Residue	Pretreatment Modification	Signal Intensity	ATH Derivatives	Peak Identification	Additional Comments
Val	None	Strong	1	Val-ATH	—
Cys	Acrylamide alkylation	Medium	2	Acrylamide-Cys-ATH dehydro-alanine-ATH	—
Ser	Acetylated and/or PIC derivatized	Low	1	Dehydro-alanine-ATH	Signal is often not seen. Can interfere with sequencing.
Thr	Acetylated and/or PIC derivatized	Medium to low	2	Diastereomers of dehydro-Thr-ATH	1 diastereomer coelutes with MNTC.
Pro	None	None	0	—	Stops sequencing.

Sequencing Cycles

Table B-3 provides a summary of the cycles used for C-terminal sequencing. The corresponding purpose of each cycle is described in the Action columns. The cycles are for initial thiohydantoin formation from a free carboxyl group, the amidation of aspartic acid and glutamic acid side-chain carboxyls, the capping of serine and threonine hydroxyl groups, and the default cycle.

Table B-3 C-Terminal sequencing chemistry overview

Cycle	Cartridge Cycle	Flask Cycle	Event or Chemistry that Occurs during the Cycle
1	Activation	Flask Prep Pump	C-terminus is converted to a thiohydantoin. 140C pumps are filled.
2	PipSCN Amidation	C-Term Flask Blank	Aspartic and glutamic acid residues are amidated with piperidine. The 140C pump runs a gradient of the flask reagents only.
3	Activation	C-Term Flask Standard	Activation is repeated as a cleanup and preparation for sequencing. The 140C pump analyzes the ATH standards.
4	OH Cap & Sequencing	C-Term Flask Normal	A modified sequencing cycle is run in the reaction cartridge. The cycle includes a brief acetylation with an acylation catalyst for capping the hydroxyl group of the serine and threonine residues.
5+	Sequencing	C-Term Flask Normal	The sequencing cycle is the default cycle, and is used for all subsequent cycles. It is comprised of alkylation steps and a cleavage/derivatization step. After cleavage, the ATH-amino acid is transferred to the flask, dried, reconstituted, and injected onto the HPLC system for analysis.

The number of residues identified using this method is sequence dependent. Typically 1 to 2 nmol of purified protein will allow sequencing of at least 3 to 5 cycles from the C-terminus. Sequencing of electroblotted samples, where less than 1 nmol of protein is present, has also been successful.

Phenyl Isocyanate (PIC) Pretreatment

Prior to C-terminal sequencing, phenyl isocyanate (PIC) pretreatment is recommended for several reasons:

- The formation of the phenylurea derivative from the epsilon amino groups of lysines increases the hydrophobicity of the protein, thereby enhancing attachment to the PVDF membrane.
- Lysine is detected as the phenylurea derivative rather than the acetylated derivative, which can be difficult to resolve from an artifact peak.
- Tyrosine is protected from acetylation by temporarily forming an arylcarbamate, which can hydrolyze back into tyrosine in the flask.
- Evidence suggests that some capping of serine and threonine hydroxyl groups occurs during PIC treatment. This can improve sequencing through these residues.

Amidation of Glutamic and Aspartic Acid Side-Chains

The carboxylic acid side-chains of aspartic and glutamic acid also react with acetic anhydride, forming mixed anhydrides. Figure B-8 portrays the formation of an oxazolone at the C-terminus, and the formation of a mixed anhydride at a glutamic acid side-chain.

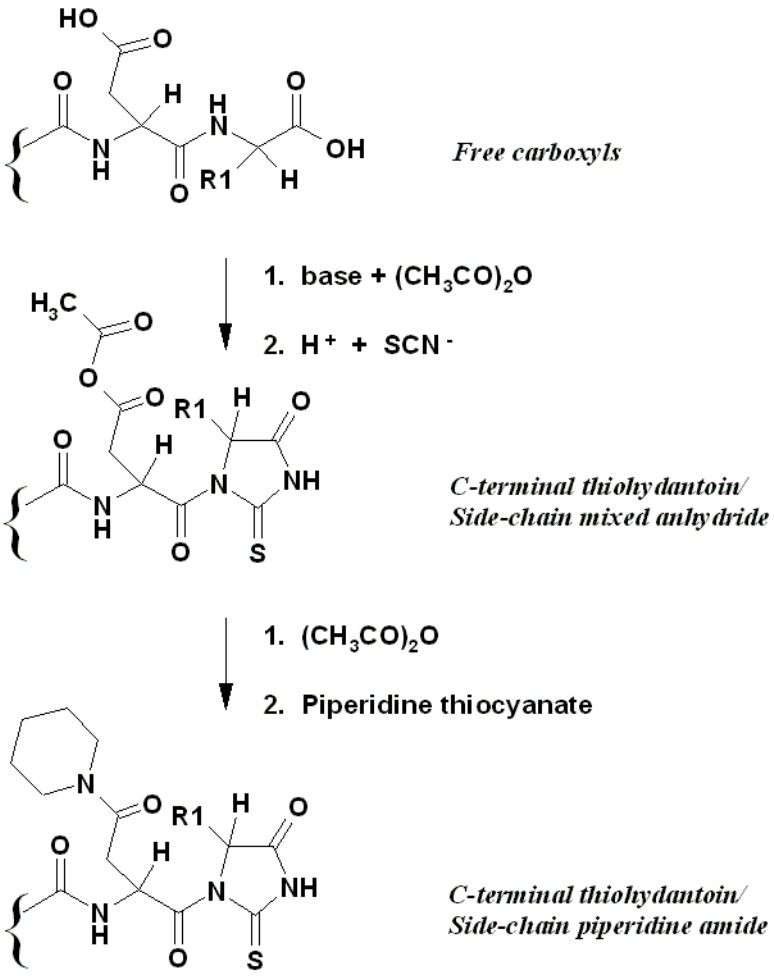


Figure B-8 Amidation of glutamic and aspartic acid

Piperidine formed by dissociation of piperidine thiocyanate was observed to react with mixed anhydrides, but not with the ionized oxazolone at the C-terminus while conditions were still basic. This results in amidation of aspartic and glutamic acid side-chains, forming the corresponding piperidine amides, which are readily detected during HPLC analysis.

Amidation of glutamic and aspartic acid side-chains is carried out preferably after proteinyl-thiohydantoin formation. However, the activation cycles and the piperidine amidation cycle have been created so that their order can be reversed.

Capping of Serine and Threonine Hydroxyls

Acetic anhydride will also acetylate the hydroxyl groups of serine and threonine. Except when serine or threonine are located at the C-terminus, the hydroxyl groups of serine and threonine interfere with the present sequencing method. Acetylation of the hydroxyl group prevents displacement of the alkylated sulfur atom of an adjacent ATH-amino acid residue during sequencing. Typically, a reduced yield is observed in cycles following a serine or threonine, even after acetylation. The ATH derivatives for serine and threonine correspond to the dehydro-analog. It has not yet been determined whether the presence of dehydrated serine and threonine in a protein prior to sequencing interferes with this method. A protein with multiple serine and threonine residues near the C-terminus remains difficult to sequence.

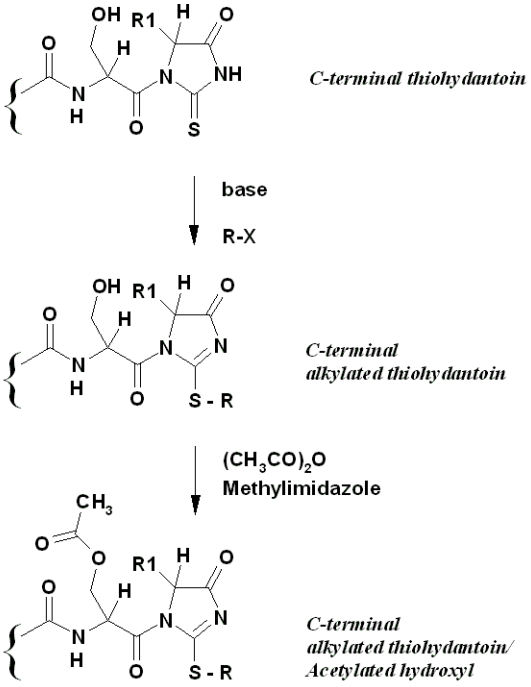


Figure B-9 Capping of serine and threonine

Efficient acetylation of the hydroxyl groups of serine and threonine requires the presence of an acylation catalyst [N-methylimidazole (NMI)], along with acetic anhydride. Because the NMI will also promote unwanted side reactions with a free carboxyl group or a thiohydantoin, capping with acetic anhydride and NMI is done after an alkylated thiohydantoin has been formed at the C-terminus. The cycle used is a modified default cycle.

Note: Some acetylation of serine and threonine will occur with acetic anhydide and a base, such as DIEA (C11). Some capping of serine and threonine hydroxyls also occurs from the use of PIC in the pretreatment.

About the Sequencing (Default) Cycle

Description

Once a thiohydantoin has been formed at the C-terminus of the protein, the alkylation of the C-terminal thiohydantoin derivative results in a derivative which is readily cleaved from the rest of the protein. Cleavage of an ATH-amino acid occurs readily with {NCS}– under acidic conditions. Cleavage of the ATH-amino acid by {NCS}– is accompanied by the formation of a new peptidyl-thiohydantoin at the C-terminus.

The need to return to a carboxylic acid functional group at the C-terminus, a group that is difficult to react efficiently and selectively, is avoided.

The cleaved ATH-amino acid is sent to the flask, where it is dried, reconstituted into 20% acetonitrile in water, and injected onto the HPLC system for analysis.

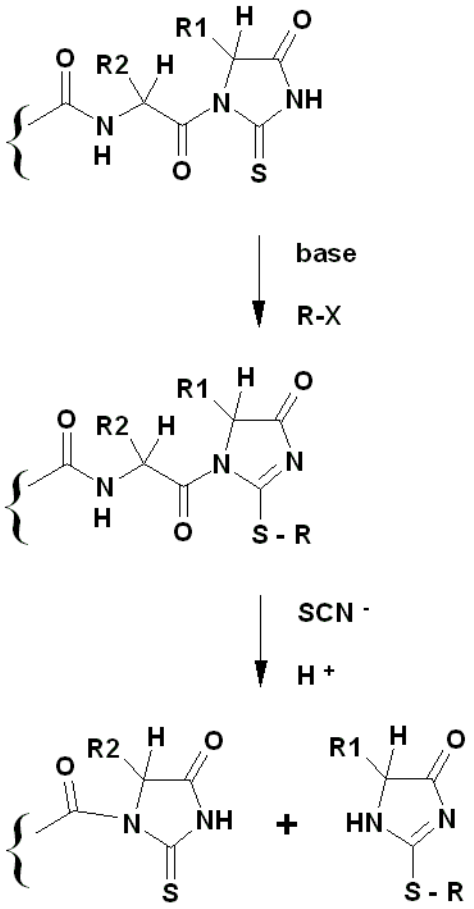


Figure B-10 The default cycle

Poor Repetitive Yield

The correct amount of TFA delivery during cleavage is critical. The tetrabutylammonium isothiocyanate must be delivered to the reaction cartridge first, followed by TFA. If TFA is delivered first, or if too much TFA is delivered, TFA will compete with {NCS}–during cleavage. If the ATH-amino acid is cleaved by TFA, derivatization into a new thiohydantoin will not occur. Therefore, too much TFA delivery is characterized by poor repetitive yield. In extreme cases, only the first cycle is obtained.

Lag

Lag is the presence of some of the previous cycle's ATH-amino acid derivative along with the expected ATH-amino acid. If lag is observed, too little TFA may have been delivered, resulting in incomplete cleavage of the ATH-amino acid. Lag will also occur if the alkylation step is incomplete, due either to insufficient base or alkylating reagent.

Preview

Preview is the presence of the next cycle's ATH-amino acid derivative along with the expected ATH-amino acid. Preview is less common than lag. The source of preview is likely from cleavage of a freshly formed thiohydantoin during prolonged treatment with {NCS}– and TFA. A thiohydantoin-amino acid *can* be cleaved under the same conditions as an ATH-amino acid, but the reaction is much slower. Preview is sequence and/or amino acid dependent. For instance, preview is occasionally associated with aspartic acid and glutamic acid residues near the C-terminus. The automated cycle that amidates aspartic acid and glutamic acid residues with piperidine greatly decreases preview.

ATH-Amino Acid Extraction and Transfer

The cleaved ATH-amino acid is transferred with ethylacetate (along with the tetrabutylammoniumisothiocyanate cleavage reagent and by-products), and dried in the flask. No additional transformations or conversion occurs in the flask for extraction.

After drying off the organic solvent, the ATH-amino acid is reconstituted in 20% acetonitrile in water, and is injected on the HPLC system for analysis. As in N-terminal sequencing, the amino acid sequence is determined by comparison of retention times to an authentic standard. The standard is injected just prior to the sequence runs. Artifacts from the chemistry are unavoidably present in the HPLC chromatograms. Optimized chromatography minimizes coelution problems.

Amino Acid Standards For C-Terminal Sequencing

Unlike the PTH-amino acid standards used in Edman sequencing, free ATH-amino acid standards are unstable in solution. To circumvent this limitation, a strategy of on-instrument generation of the standards was devised. The standard mixture is provided with t-Boc protected alpha-amino groups. During the ATH-amino acid standard cycle, an aliquot of standard is delivered to the flask and dried down. Neat TFA is delivered to the flask to remove the t-Boc protecting groups. The TFA is dried and the standard reconstituted in 20% acetonitrile in water.

The Boc-protected ATH-amino acid standards were synthesized from Boc amino acids. A quantitative solution of the purified materials in anhydrous acetonitrile can be stored on-instrument for an extended period without degradation.

Sample Transfer and Injection onto the Column

Once the sample has been reconstituted in the flask, it is transferred to the HPLC injector loop. Transfer is accomplished by pressurizing the flask with argon, and driving the sample out through the pick-up line into the injector loop. When the sample loop load sensor detects fluid, the injector valve is switched from the load to the inject position, moving the sample into the HPLC solvent stream. The gradient program and data collection begin.

Flask cycles must include the following steps:

Step	Description
Function 227, Prepare Pump	Gradient information is downloaded to the 140C pump from the SequencePro software. After downloading is complete (30 to 60 sec), the 140C pump will start, pressurize and run at the initial gradient conditions.
Function 226, Load Position	This function puts the injection valve in the load position. It must precede the Load Injector step for the sample loop to be flushed before sample transfer to the sample loop.
Function 221, Flush Injector	Flushes the sample loop from valve 44. Does not flush through the flask. This function must precede the Load Injector step.
Function 225, Load Injector	Activates the sample loop sensors. Transfers sample from the flask to the HPLC sample loop.

Valves, Functions, Cycles, and Methods

C

This chapter describes the types of valves, and the purpose of functions, cycles, and sequencing methods used by the PROCISE® Protein Sequencing Systems.

The following topics are contained in this appendix:

Important Terms for Users

Table of Terms

A clear understanding of the following terms is important for the proper use of this system:

Term	Description
Valve	A mechanical device that opens and closes to provide a flow path for the transfer of gas, solvent, or reagent. There are three types of valves: • Delivery valves for liquid, vapor or gas delivery • Three-way valves for gas delivery only • Bottle vent valves for venting chemical bottles
Function	A function: • Activates a valve or set of valves to deliver a chemical. • Activates or deactivates a relay. • Defines or increments a setpoint. • Directs the 140 pump to start and stop. • Can have a fixed or global time associated with it. • Can be customized. Refer to “Creating and Modifying Functions” on page 5-2 in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients.” for information on creating functions.
Step	A function that has been incorporated into a cycle. Steps are the building blocks of cycles.
Cycle	A series of steps that accomplishes a specific chemical process in the reaction cartridge or flask. • Can be customized. Refer to “Modifying Cycles,” in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients,” for information on customizing cycles.
Method	A group of cycles designed to sequence a peptide or protein. Typically begins with one cycle, followed by several repetitions of another cycle. • Includes starting temperatures for the cartridge, flask, and column. • Includes the gradient program to be run by the 140 pump. • Can be customized. Refer to “Modifying Methods,” in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients,” for information on customizing methods.
Gradient	A programmed run for the HPLC components of the system that defines flow rate and solvent composition changes over a specified period of time. • Downloaded to the 140 pump via the computer. • Can be customized. Refer to “Gradient Programming Guidelines” on page 5-12 in Chapter 5, “Custom Functions, Cycles, Methods, and Gradients,” for information on customizing gradients.

Purpose and Types of Valves Inside the Sequencer

There are several types of valves that control gas and chemical deliveries inside the sequencer:

- Three-way valves
- Bottle vent valves
- Delivery valves

Valves are electronically opened (activated) and closed (deactivated) to create pathways to a particular destination, such as a reaction cartridge.

Each valve is assigned a number. The valve diagram on page C-5 illustrates the position of each valve.

Three-Way Valves

Three-way valves are used exclusively for argon delivery. Three-way valves:

- Control argon input to valve positions 15, 24, and 44
- Provide two different argon pressures (high and low) from the same manifold inlet line

Table C-1 Standard Pressures for Three-Way Valves

Valve Status	Function(s)	Pressure
Valve 46 off	Cart dry	3.5 psi
Valve 46 on	All cart block flushing	Internal manual regulator pressure
Valve 47 off	Flask dry; all flask flushing	3.0 psi
Valve 47 on	Load injector	0.8 psi
Valve 48 off	Flask bubble; low pressure sample loop flushing	1.8 psi
Valve 48 on	High pressure sample loop flushing	Internal manual regulator pressure

Bottle Vent Valves

The protein sequencer uses bottle vent valves to control the flow of argon. Argon is required for bottle pressurization and flushing.

- There is one bottle vent valve for each chemical bottle, making a total of twelve bottle vent valves.
- The valves are activated by the pressure control system to maintain proper bottle pressurization.

During chemical delivery, the bottle vent valves remain closed. During venting or flushing, the valves are opened. This allows the argon in the bottle headspace to flow to waste.

Delivery Valves

Delivery valves are grouped into valve blocks. Seven valve blocks, connected with Teflon tubing, comprise the chemical delivery system.

Delivery Valve Block	Description
Cartridge Reagent Block	Controls delivery of the reagents: • R1 • R2 • X1 (liquid and gas) • X3 to the cartridge input block and to waste.
Cartridge Solvent Block	Controls delivery of: • Reagent R3 (liquid and gas) • Solvents S2B, S3, and S1 • Argon to the cartridge reagent block, cartridge input block, cartridge output block, and to waste.
Cartridge Input Block	Controls the transfer and metering of reagents, solvents, and argon from: • The cartridge reagent block • The cartridge solvent block into or out of the active cartridge, and to waste.
Cartridge Output Block	Controls the transfer and metering of reagents, solvents, and argon from: • The cartridge reagent block • The cartridge solvent block into or out of the active cartridge, and to waste.
Flask Reagent Block	Controls the delivery and metering (small loop) of: • Reagents R4, R5, X2 (liquid), X3 • Solvent S4C • Argon to the flask input block.
Flask Input Block	• Controls the delivery of X2 (gas.) • Controls the transfer and metering (large loop) of reagents, solvents, and argon from the flask reagent block to the conversion flask and to waste.
Flask Output Block	• Controls the delivery of argon to the conversion flask for bubbling and for flushing the sample loop • Controls the transfer of the conversion flask contents to the sample loop and to waste.

Valve Design

The design of the valve blocks minimizes any holdup volume following chemical delivery.

Delivery lines feed into each valve block, and connect to the common pathway (manifold) inside the block through a manifold inlet line and a solenoid-controlled valve. Delivery from the inlet line into the manifold occurs only when the appropriate valve is activated. The manifold zigzags through the valve block to bypass closed valves.

The direction of the flow is determined by the pressures on both sides of the pathway.

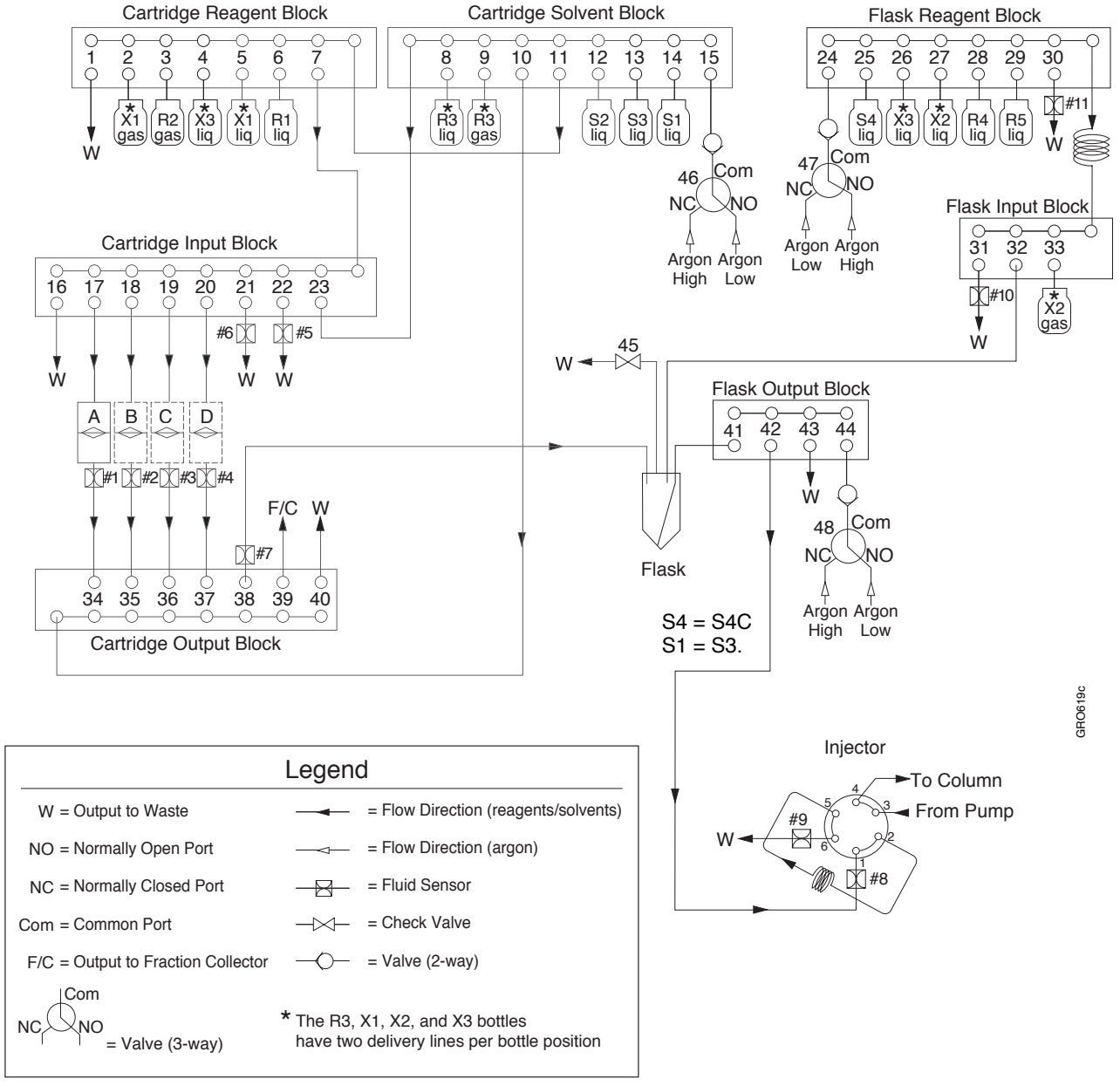


Figure C-1 Procise cLC Protein Sequencer valve diagram

Purpose and Types of Functions

Overview of Functions

Functions are the building blocks of cycles. Each step in a cycle is a function.

In general, functions are used to:

- Activate and deactivate the valves inside the sequencer
- Activate the sensors that control valve operation
- Signal the start and end of a cycle
- Facilitate the transfer of a sample from the reaction cartridge to the flask, and from the flask to the column
- Set temperatures for the cartridge, flask, and column
- Download and start the gradient program run by the 140 pump

Function types

The information on pages C-6 through C-9 describes each of the following function types:

- Valve control functions
- Fluid sensor functions
- Cycle-synchronizing functions
- System functions
- User-defined functions

Function Grouping and Numbering Schemes

A function grouping and numbering scheme (Table C-2 on page C-6) simplifies the programming and operation of the PROCISE cLC protein sequencing system.

Each function is assigned:

- A name that describes its purpose
- A number based on the function grouping

To view available functions, open the Functions dialog box from the dialog box pop-up menu on the computer.

Note: A list of the standard functions supplied with this system is located in Appendix A.

Function Numbers

The table below lists the numbering scheme and grouping of functions.

- Standard functions are numbered 1–400
- Numbers 401–450 are reserved for user-defined functions

Table C-2 Function grouping and numbering formats

Standard Function numbers:	
1–150	Cartridge functions
151–250	Flask and HPLC functions
251–259	Cartridge and flask transfer functions
260–360	Cartridge and flask test and procedure functions

Table C-2 Function grouping and numbering formats *(continued)*

361–400	Cartridge and flask reserved
401–450	Reserved for user-defined functions

Valve Control Functions

Valve control functions activate (open) and deactivate (close) a valve or a set of valves. The valves are opened simultaneously to deliver a chemical or gas.

Fluid Sensor Functions

Fluid sensor functions control the activation and deactivation of valves using fluid sensors.

Fluid sensors control certain valves inside the sequencer.

When a sensor function begins, the fluid sensor tries to detect fluid such as reagent or solvent. When fluid is detected, the reagent or solvent delivery valve is turned off-hour the injector is triggered to switch positions.

The duration of a sensor function must be long enough for fluid to reach the sensor. The remaining time allotted for the function continues to count down to zero, then the next step begins.

If fluid does not reach the sensor by the end of the step, an error message is sent to the Event Log file, and sequencer operation is paused.

Cycle-Synchronizing Functions

A group of functions are used to provide proper synchronization of the cartridge and flask cycles during sequencing. Every cycle must have Begin (Function 258) and End (Function 259) steps in order to be valid. The flask or the cartridge cycle will wait at the Begin step, if necessary, in order to keep the cycles synchronized. The cycle timer will count up (increment) during this step if a cycle is synchronizing. At the appropriate time, the cycle will automatically proceed to the next step.

The timing for transfer from cartridge to flask is defined by a set of steps in the cartridge cycle and one step in the flask cycle. A Ready to Receive (Function 228) step in the flask cycle indicates that the flask is ready to accept sample transfer from the cartridge. The flask will wait at this step, with the cycle timer incrementing, until the transfer is complete.

The beginning and end of the transfer from the cartridge to the flask are defined in the cartridge cycle respectively by, the steps Ready Transfer to Flask (Function 127) and Transfer Complete (Function 128). Synchronization is set up in such a way that the Ready to Receive step in a flask cycle occurs 5 seconds before the Ready to Transfer step in the cartridge cycle.

System Functions

Function Name	Number	Description
Set Cart Temperature	142	Used to adjust the cartridge temperature at a fixed time during a cycle. Acceptable temperature range: ambient to 70 °C.

Table C-3 Flask System Functions

Function Name	Number	Description
Load Position	226	Switches the sample loop out of the HPLC flow path. During a flask cycle, this function must precede the Load Injector step in order for the sample loop to be flushed and the sample in the flask to be transferred into the sample loop.
Set Flask Temperature	230	Used to adjust the flask temperature at a fixed time during a cycle. Acceptable temperature range: ambient to 78 °C.
Prepare Pump	227	Downloads gradient information from the PROCISE cLC software to the pump. After downloading is complete (30–60 sec.), the pump will start, pressurize, and run at the initial gradient conditions.
Stop Pump	231	Stops any pump activity.
Start Gradient	232	Used to start the gradient in cases where there will be no sample injected.
Inject Position	223	Switches the sample loop into the HPLC flow path. Not necessary when using the Sample Loop Load sensor. When the sensor detects fluid, the Rheodyne valve is automatically activated.
Set Column Temperature	229	Used to adjust the column temperature at a fixed time during the flask cycle. Acceptable temperature range: ambient to 70 °C.

Table C-4 Common System Functions

Function Name	Number	Description
Begin	258	This function must be the first step of all cycles, tests, and procedures.
End	259	This function must be the last step of all cycles, tests, and procedures.
Wait	257	This function is used to keep the cycle time running for a particular step in a cycle while all the valves are closed.

User-Defined Functions

You can create your own functions for specialized needs or applications. Refer to Chapter 5, “Custom Functions, Cycles, Methods, and Gradients,” for information on creating your own functions.

Specifying Valve Function Times

Valve-control functions are referred to as time-dependent functions because the valves are opened for a fixed period of time, then closed. The time is specified:

- As a parameter in a cycle
- By the operator via manual control mode

Valve Flow Path Diagram

To trace the flow path created by a valve-control function, refer to the valve diagram in this manual (Figure C-1 on page C-5).

The valve diagram is also on the operator assistance card inside of the front cover of the sequencer.

Purpose and Types of Cycles

A cycle is designed to control the chemical processes that must occur to sequence a protein or peptide. A cycle is a group of functions.

- Once incorporated into a cycle, each function becomes a step in that cycle.
- Steps are activated for a specific period of time during the cycle.

Types of Cycles

There are two types of cycles supplied by Applied Biosystems for use with the protein sequencing system:

- Standard cartridge cycles
- Standard flask cycles

Note: Standard cycles cannot be deleted or modified, but can be used as templates for creating new cycles. Refer to Chapter 5, "Custom Functions, Cycles, Methods, and Gradients," for information on creating cycles.

Sequencing Methods

A method combines a number of cartridge cycles, flask cycles, HPLC gradients and operating temperature settings in the order necessary to perform a specific task such as the analysis of a protein sample. There are sequence methods for preparing glass fiber filters prior to sample loading, methods specific to the sample substrate and the type of cleavage employed and methods for testing or optimizing the sequencer performance.

Warranty and Service Information

D

This appendix describes the PROCISE®, PROCISE® cLC and PROCISE® C Protein Sequencing Systems instrument warranty.

The following topics are contained in this appendix:

Computer Configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation.
Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited Product Warranty

Limited warranty

Applied Biosystems warrants that all standard components of its PROCISE, PROCISE cLC, or PROCISE C Protein Sequencing System will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its PROCISE, PROCISE cLC, or PROCISE C Protein Sequencing System such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins.
Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media.

Applied Biosystems does not warrant that the operation of the instrument or its operating software will be uninterrupted or error free.

Warranty period effective date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer.

Warranty exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems' sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed.

Warranty limitations

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, tort, warranty, or under any statute (including without limitation, any trade practice, unfair competition, or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person or entity, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damages, including without limitation, damages arising from or related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business, or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to modify the terms of this Limited Warranty Statement or to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement, and any such modification, affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems, unless in a writing signed by an executive officer of Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, Claims, Returns

Damages

If shipping damage to the PROCISE, PROCISE cLC, or PROCISE C Protein Sequencing System is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is received by Applied Biosystems, Applied Biosystems will process the claim unless other instructions are provided.

Returns

Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems representative.

Index

Symbols

Numerics

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

Worldwide Sales and Support
Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at www.appliedbiosystems.com.

www.appliedbiosystems.com

Applied Biosystems

Applera Corporation is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Printed in USA, 02/2003
Part Number 4340645 Rev. A

an **Applera** business