Expedite™ 8900

Nucleic Acid Synthesis System

User's Guide

PerSeptive Biosystems, Inc.
500 Old Connecticut Path
Framingham, MA 01701 USA

Part Number 601306, Rev. 1
March 1997

NOTICE

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Nucleic acid synthesis reagents sold by PerSeptive Biosystems, Inc. are covered by U.S. patent RE34,069 and patents in Austria, Belgium, Canada, France, Germany, Japan, Luxembourg, Netherlands, Sweden, Switzerland, and U.K.

Printed on recycled paper.

WARNING

For continued protection against fire hazard, replace fuses with those of the same type and rating.

AVERTISSEMENT

Remplacez les fusibles par ceux de même type et puissance pour éviter les risques d'incendie.

WARNING

Most of the reagents and solvents used in nucleic acid synthesis are hazardous. Wear a lab coat, gloves and eye protection when handling reagents. Adequate ventilation is essential and working under a fume hood is recommended. Consult Appendix B, Reagent Safety, for reagent safety considerations.

AVERTISSEMENT

La plupart des réactifs et solvants employés en synthèse d'acides nucléiques sont dangereux. Portez une blouse, des gants et des lunettes de protection lorsque vous manipulez des réactifs. Une ventilation adéquate est nécessaire et il est recommandé de travailler sous une hotte. Consultez la partie "Appendix B, Reagent Safety", de ce manuel.

US Safety and EMC (Electromagnetic Compliance) Standards

Safety

This instrument has been tested to and complies with standard ANSI/UL 1262, “Electrical Equipment for Laboratory Use; Part 1: General Requirements”, 1st Edition. It is an ETL Testing Laboratories listed product.

EMC

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

***Warning**: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.*

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Note: Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.

Canadian Safety and EMC (Electromagnetic Compliance) Standards

Safety

This instrument has been tested to and complies with standard C22.2 No. 151, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements”. It is an ETL Testing Laboratories listed product.

Sécurité

Cet instrument a été vérifié avec la norme C22.2 No. 151, «Spécifications de sécurité du matériel électrique utilisé pour les mesures, les contrôles et dans les laboratoires ; Partie 1 : Spécifications générales», et il est conforme à cette norme. C’est un produit homologué par les ETL Testing Laboratories.

EMC

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le materiel brouilleur du Canada.

European Safety and EMC (Electromagnetic Compliance) Standards

Declaration of Conformity

Application of Council Directive(s):	73/23/EEC “Low Voltage”
	89/336/EEC “Electromagnetic Compatibility”
Standard(s) to which conformity is declared:	IEC 1010-1 “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use”
	EN55011:1991, Group 1, Class A “Radiated Emissions”
	EN50082-1:1991 “Generic Immunity”
Manufacturer’s Name:	PerSeptive Biosystems, Inc.
Manufacturer’s Address:	500 Old Connecticut Path
	Framingham, Massachusetts 01701 USA
Type of Equipment:	Laboratory Instrumentation
Model Name & Number:	Expedite Nucleic Acid Synthesis System, Models 8905 and 8909
Part Number:	GEN600041 or GEN600042
Serial Number:	FX???8200? and later Question marks represent date and manufacturing codes that are part of the serial number.
Year of Manufacture:	1995 and later

Table of Contents

Chapter 3 Software Reference

Chapter 4 Maintenance and Troubleshooting

How to Use This Guide

Purpose of this guide

The PerSeptive Biosystems' *Expedite™ 8900 Nucleic Acid Synthesis System User's Guide* describes the features and use of the Expedite Nucleic Acid Synthesis System. It also includes routine maintenance and troubleshooting procedures.

Structure of this guide

PerSeptive Biosystems' *Expedite 8900 Nucleic Acid Synthesis System User's Guide* is divided into chapters. Each chapter page is marked with a tab and a header to help you locate information within the chapter.

The table below describes the material covered in each chapter.

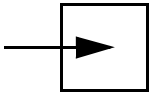
Chapter 1, The Expedite Nucleic Acid Synthesis System	Describes the main components of the instrument.
Chapter 2, Performing a Synthesis	Describes how to set up and run a synthesis.
Chapter 3, Software Reference	Describes the structure and operation of the software.
Chapter 4, Maintenance and Troubleshooting	Contains routine maintenance procedures and some simple troubleshooting procedures.
Appendix A, Installation	Contains installation procedures.
Appendix B, Reagent Safety	Includes chemical handling precautions

Appendix C, Performance Specifications	Lists performance specifications.
Appendix D, Nucleic Acid Synthesis Reagents	Lists part numbers and descriptions for reagents you need to run the system.
Appendix E, Accessories and Spare Parts	Lists part numbers you need to order parts and accessories from PerSeptive Biosystems.
Appendix F, Trityl Monitor	Describes using the trityl monitor.
Appendix G, Warranty/Service	Contains warranty, service, return, and spare parts information.

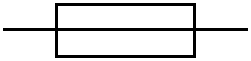
Symbols used on the instrument

The following symbol is used on the Expedite Nucleic Acid Synthesis System to instruct you to refer to the User's Guide for more information.

The following symbol is used to show where gas pressure enters the instrument. The maximum inlet pressure is 25 psi.



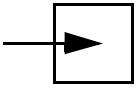
The following symbol is used to show the values of the fuses required by the instrument.



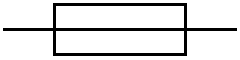
Symboles Utilisés sur cet Appareil

Sur le synthétiseur d'acides nucléiques Expedite, lorsque vous rencontrez le symbole suivant, reportez-vous au Guide de l'utilisateur pour de plus amples informations.

Le symbole suivant indique l'endroit où le gaz sous pression entre dans l'appareil. La pression d'entrée maximale est de 25 psi (1,7 bar).



Le symbole suivant indique le type de fusibles nécessaires au fonctionnement de cet appareil.



Conventions

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

General conventions

- **Bold** indicates user action:

  "Type **0** and press **Enter** for the remaining fields."

- *Italic* text denotes new or important words, and is also used for emphasis:

  "Before analyzing, *always* prepare fresh matrix."

Notes, Cautions, and Warnings

A note calling out important information to the operator appears as:

***NOTE**: Record your result before proceeding with the next step.*

A caution calling out information to avoid damage to the system or equipment appears as:

CAUTION

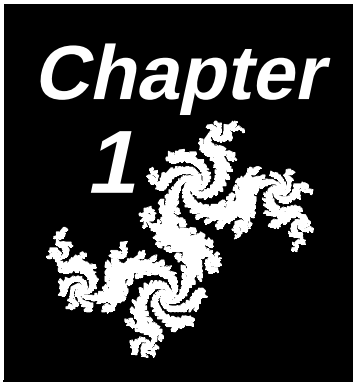
Changing reagent bottles during a synthesis is not recommended. Check the reagent resources prior to initiating a synthesis to make sure that there is a sufficient supply.

A warning calling out information essential to the safety of the operator appears as:

WARNING

The Expedite Cabinet weighs 102 pounds (46 kg). Two people are required to safely lift the instrument cabinet.

1 The Expedite™ Nucleic Acid Synthesis System



This chapter contains the following sections:

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1.1 Introduction

The Expedite™ Nucleic Acid Synthesis System, shown in Figure 1-1, is a dual column instrument that is versatile and easy to operate.

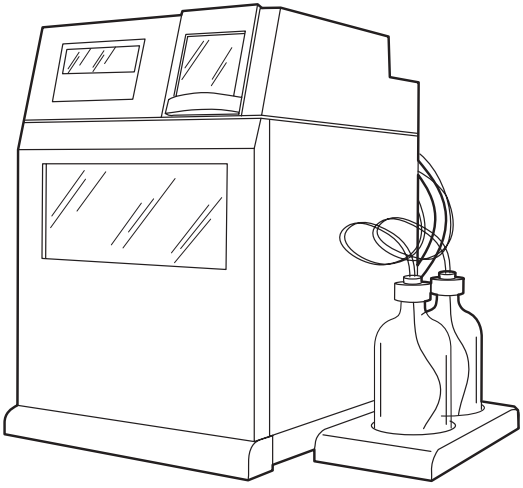

Figure 1-1 Expedite Model 8909 Nucleic Acid Synthesis System

The Expedite Nucleic Acid Synthesis System features:

- Alternating phase dual column synthesis
- Low reagent consumption
- A full range of DNA and RNA synthesis protocols
- Wide range of synthesis scales
- A choice of vessel configurations
- Microfluidic delivery system
- Trityl monitor
- Optional accessories

Dual Column Synthesis

The two columns can operate independently allowing the simultaneous synthesis of two different sequences at different scales. At any time, a second synthesis may be started or an individual synthesis may be paused or aborted without affecting concurrent operations on the other column.

Low Reagent Consumption

The total reagent consumption is less than 4.5 mL per coupling cycle for scales up to 1 μmole. The phosphoramidite consumption is less than 3 mg (~50 μL) per coupling cycle at the 0.05 μmole scale.

The Model 8909 can perform 800 coupling cycles (0.2 μmole scale) without replenishing the reagents and the Model 8905 can perform 150 coupling cycles at the same scale without replenishing the reagents.

Protocols

The following protocols are available on the Expedite Nucleic Acid Synthesis System:

- β-Cyanoethyl phosphoramidite DNA synthesis at 0.05 μmole, 0.2 μmole, 1 μmole, and 15 μmole scales
- β-Cyanoethyl phosphoramidite RNA synthesis at 1 μmole scale
- β-Cyanoethyl phosphoramidite synthesis of phosphorothioated DNA at 1 μmole and 15 μmole scales

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***NOTE**: You can synthesize phosphorothioated DNA in other scales by using a DNA synthesis protocol and changing the upper case base designations to lower case base designations.*

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The 8900 Series

The PerSeptive Biosystems Expedite Nucleic Acid Synthesis System provides a choice of two models:

- Model 8905
- Model 8909

The Model 8905 has five nucleotide monomer reservoirs and six ancillary reagent reservoirs with a total capacity of 150 cycles at 0.2 µmole scale.

The Model 8909 offers greater flexibility and increased reagent capacity with nine nucleotide monomer reservoirs and eight ancillary reagent reservoirs with a total capacity of 800 cycles at 0.2 µmole scale. The additional reservoirs may be used for a variety applications such as:

- Phosphorothioated nucleic acid fragments and applications using nonstandard monomers
- Multiple fluorescent labels for probes and sequencing
- Mixed hybridization probes
- Linkers (3' and 5')
- RNA and DNA Hybrids

Trityl Monitor

The trityl monitor detects the DMT (dimethoxytrityl) group as it is removed during the deblocking step. During synthesis, the trityl information is displayed as a histogram on the instrument display or workstation and can be sent later to a printer. In the event of a failure in the coupling chemistry, the trityl monitor can also be set to suspend a synthesis.

The trityl monitor includes a waste diversion system that separates the non-chlorinated waste from the chlorinated waste for safe and economical disposal of the chlorinated waste.

Interface cables are available for automatic advance of an Isco brand fraction collector.

1.2 Accessories

The following accessories are available for purchase with the basic Expedite Nucleic Acid Synthesis System:

- Expedite Workstation software
- Multiple Oligonucleotide Synthesis System (MOSS) option (8909 systems only)
- Expedite PNA Instrument option
- Uninterruptable Power Supply (UPS)

Refer to Appendix E, Accessories and Spare Parts, for part numbers.

Expedite Workstation Software

The Expedite Workstation software provides an external user interface to one or more instruments. The Microsoft® Windows®-based package is a complete multiple instrument management system that includes:

- Synthesis monitoring and system control
- A sequence editor with the capability to import data from third party DNA software
- A synthesis protocol editor
- A report generation facility
- An internal database for managing sequences, protocols, and reports

The Expedite Workstation software requires a minimum system configuration of:

- PC compatible 386SX 16MHz computer
- 4 MB RAM minimum, 8 MB RAM recommended
- 60 MB Hard Drive
- 3.5” Floppy Disk Drive
- VGA color graphics monitor
- MS-DOS® Version 5.0
- Microsoft Windows Version 3.1
- RS-422 Serial Interface Card

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MOSS option (8909 systems only)

The Multiple Oligonucleotide Synthesis System (MOSS) option expands the capabilities of 8909 Expedite systems to allow unattended synthesis of 16 oligonucleotides. Expedite systems configured with the MOSS option allow interactive trityl monitoring for all 16 column positions. The MOSS unit sits on top of the Expedite system. It does not require additional bench space.

PNA instrument option

The PNA instrument option includes the software upgrade and reagents you need to synthesize PNA (peptide nucleic acid) oligonucleotides. The option kit includes reagents for 150 cycles. This option does not require any changes to the hardware.

UPS Power Supply

A UPS (uninterruptable power supply) is a high-performance standby that protects the instrument from utility line failures that could result in loss of syntheses.

If a utility failure occurs, such as a blackout, brownout, or sag, the UPS rapidly transfers the instrument that is plugged in at its output to an alternative power source. The alternative power source is derived from a rechargeable battery within the UPS and provides you with up to 30 minutes to continue the synthesis to a chemically safe stopping point. You can configure the stopping point in a user profile.

Two UPS options are available from PerSeptive Biosystems:

- 115 Vac
- 220 Vac

1.3 Instrument Description

The Expedite Nucleic Acid Synthesis System is a fast, flexible instrument with a small footprint. The Series 8900 instruments have the following components:

- Software Control System
- Reagent Delivery System
- Pneumatic Control System
- Power Supply

1.3.1 Software Control System

The Expedite Nucleic Acid Synthesis System is controlled by menu driven software which:

- Allows independent single or dual column operation.
- Provides full sequence editing and storage facilities. The system can store 504 sequences of up to 250 bases in length.
- Enables the synthesis of mixed sites.
- Calculates reagent requirements and monitors reagent consumption for the synthesis.
- Monitors the synthesis and displays the current status and estimated time of completion.
- Provides extended diagnostics for troubleshooting.
- Provides software tools for priming the instrument and various manual functions.
- Monitors the system for error conditions.

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- Allows multiple user profiles for individualized operation.
- Generates a log of all activities.
- Can generate a hard copy of synthesis information.
- Reminds you, after an idle period, to prime the reagent passages before you start a synthesis.

User Interface

The user interface consists of an electroluminescent backlighted LCD display with a membrane keypad. The membrane keypad, which is located below the display, has eight soft (software defined) keys and a dedicated Stop key (see Figure 1-2).

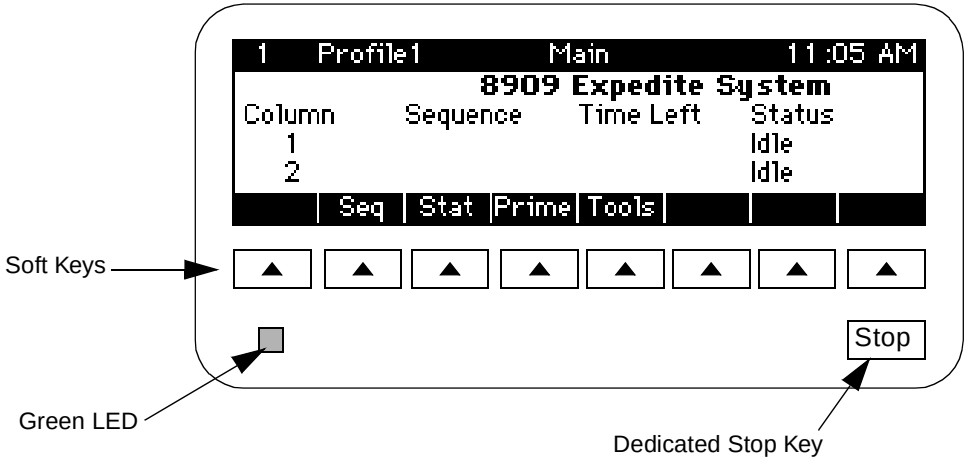

Figure 1-2 LCD Display

Different functions are assigned to the soft keys in each menu. The soft key assignment is displayed at the bottom of each screen. Menu selections are made by pressing the soft key directly below the desired selection.

In the 8900 Series, the menu structure is hierarchical and is optimized to minimize keyboard interaction.

Screen Saver

The automatic screen saver blanks the screen after the instrument has had no keypad interaction for two hours. The green LED at the left side of the keypad flashes while the screen is blanked. The screen display is restored whenever you press a key on the keypad.

Sequence Entry

You may enter sequences in the Sequence mode. Sequences are stored in user profiles. Each profile can store 63 sequences of up to 250 bases in length.

***NOTE**: Sequence entry is case-sensitive:*
DNA protocol: uppercase=DNA, lowercase=Thio
Thio protocol: uppercase=Thio, lowercase=DNA
RNA protocol: uppercase=RNA, lowercase=DNA

Sequence entry is described in Section 3.3.1, Using the Sequence Editor.

Mixed Sites

You may incorporate mixed sites into a sequence (see "Mixed Site Entry" on page 3-16). According to the mixture you enter, an equal volume of each monomer solution is delivered to the reaction column from the nucleotide reservoirs.

Reagent Requirements

The software automatically calculates the reagent requirements for a selected synthesis and monitors reagent consumption during the synthesis.

You may use the Status Resources display, at any time during a synthesis, to see how much reagent is currently estimated to be left in the reagent reservoir. See Section 3.4.1, Displaying System Information.

***NOTE:** If you rely on the instrument to report reagent consumption, you must use the bottle tool to reset the reagent volumes whenever you replenish the reagent reservoirs. See* Section 3.6.2, Bottle Change Tool*.*

Synthesis Monitoring

The software monitors the instrument during a synthesis and keeps track of the steps in the synthesis and the status of the instrument. See Section 3.4, Status Menu.

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Priming Tools

The priming tools provide options for manually priming the instrument and performing post synthesis operations. See Section 3.5, Prime Menu.

Diagnostic Tools

The software provides extended diagnostics for troubleshooting. See Section 3.6.1, Diagnostic Routines.

User Profiles

The software allows you to set up eight user profiles for individualized operation. In a user profile, you may specify default running parameters and protocols that are in effect for every synthesis that is run when that user profile is selected.

Instrument Log

The software keeps track of all information about a synthesis and automatically stores it in the instrument log on the floppy disk. If a printer is attached you may generate a hard copy of the log.

Power Failure Recovery

In the event of a power failure, the system knows where the synthesis was interrupted and, if Auto Restart is activated in the User Profile, can continue the synthesis at that point when the power is restored.

If the instrument is equipped with an uninterruptable power supply, you may specify defaults in the User Profile that minimize the damage to a synthesis if there is a problem with the electrical power (see Section 3.6.6, Specifying a User Profile). In the event of a power failure, the UPS can provide power to the instrument until the next chemically safe stopping point (end of current cycle) in the synthesis.

Printer

A dot matrix printer may be attached to the instrument through the parallel port on the back panel of the instrument. You may print:

- Sequence information
- The resources calculation
- The software configuration
- The instrument log
- Trityl data

1.3.2 Reagent Delivery System

WARNING

Most of the reagents and solvents used in nucleic acid synthesis are hazardous. Wear a lab coat, gloves and eye protection when handling reagents. Adequate ventilation is essential and working under a fume hood is recommended. Consult Appendix B, Reagent Safety for reagent safety considerations.

AVERTISSEMENT

La plupart des réactifs et solvants employés en synthèse d'acides nucléiques sont dangereux. Portez une blouse, des gants et des lunettes de protection lorsque vous manipulez des réactifs. Une ventilation adéquate est nécessaire et il est recommandé de travailler sous une hotte. Consultez la partie "Appendix B, Reagent Safety", de ce manuel.

The reagent delivery system is composed of:

- Fluid Transport System
- Reagent Reservoirs
- Reaction Columns
- Waste System
- Drip Trays

Fluid Transport System

The fluid transport system consists of a microfluidic plate with fluid passages, valve actuators, and fluid injectors. The fluid injectors are driven by the pneumatic system. Flow paths are created within the plate by the activation of specific valves.

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Each reagent is delivered as fixed volume pulses by individual fluid injectors. Therefore, consistent reagent volumes are delivered to the reaction columns. This enables accurate monitoring of reagent usage without calibrating the flow rates for each reagent.

There are two valve trains (see Figure 1-3) that deliver reagents to the reaction columns.

- The A-train delivers the deblocking, washing, capping and oxidation reagents.
- The B-train delivers the activation and coupling reagents.

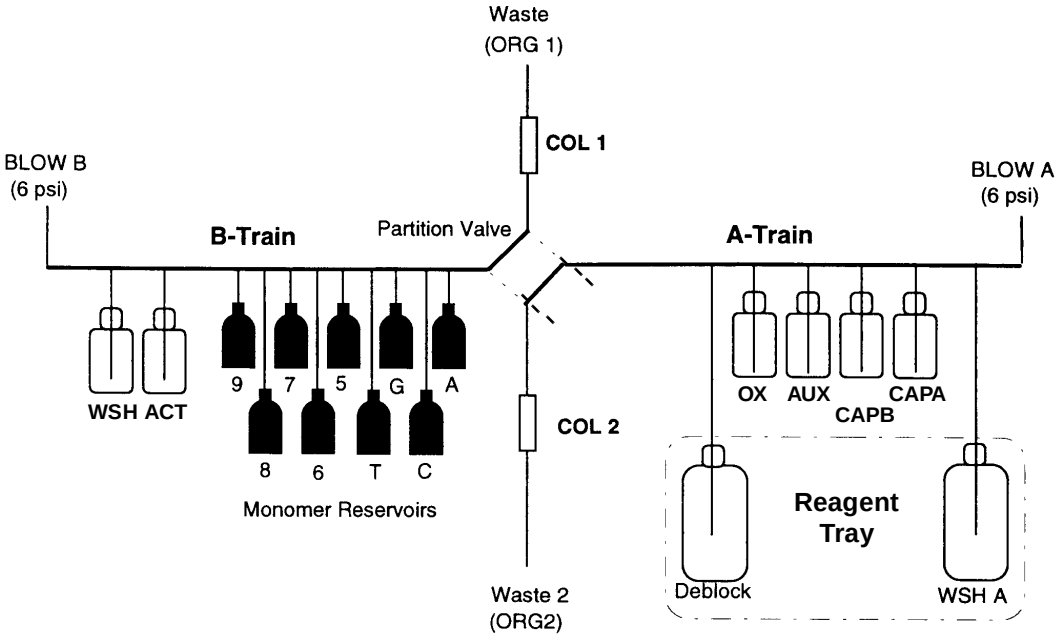

Figure 1-3 Fluidics Diagram for Model 8909

In dual column mode, the A-train delivers reagents to one reaction column at the same time that the B-train is delivering reagents to the other reaction column. Thus, two different protocols can be performed simultaneously.

Reagent Reservoirs

The reagent reservoirs screw into the positions within the instrument cabinet (see Figure 1-4). The reservoir positions are labeled to correspond to the labels of the bottles in the reagent kit. The bottles have transparent labels that display both the reservoir position on the instrument and the reagent name as it appears in the software. For a successful synthesis, the reagents must be loaded in the correct positions.

Réservoirs à Réactifs

Les réservoirs à réactifs doivent être positionnés en les vissant à certains endroits à l'intérieur de l'appareil (voir Figure 1-4). Les différents emplacements sont étiquetés de la même manière que les différents flacons du kit de réactifs. Les flacons sont munis d'étiquettes transparentes indiquant à la fois la position du réservoir dans l'appareil et le nom du réactif tel qu'il apparaît dans le logiciel. Pour réussir la synthèse, il convient de charger correctement ces réactifs.

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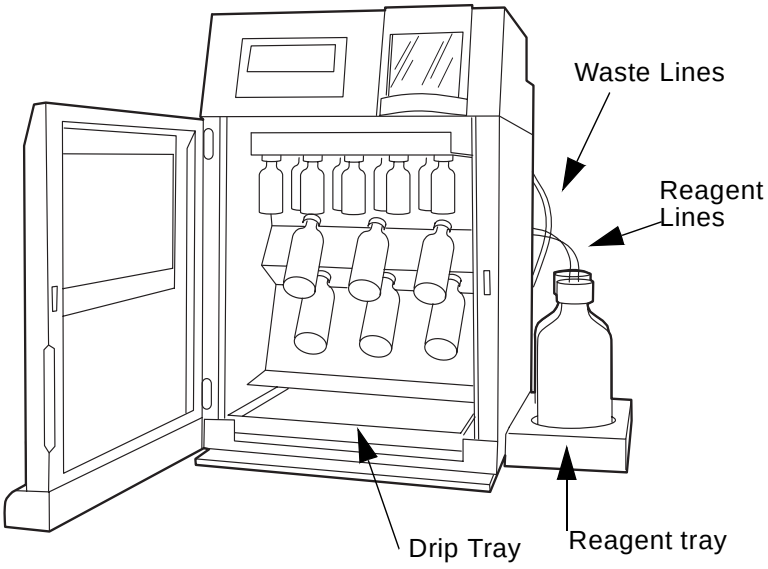


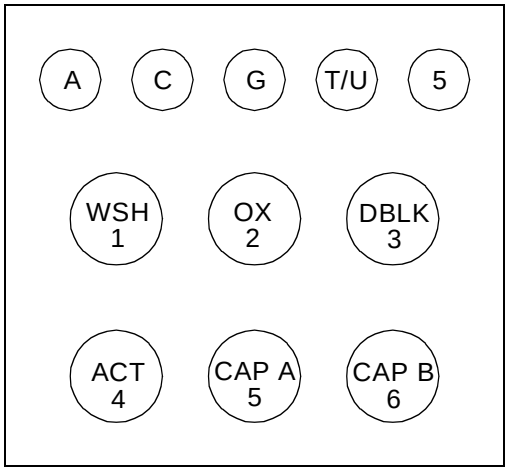
Figure 1-4 Model 8909 Instrument Cabinet with Reagent Bottles

Model 8905 Reagent Reservoirs

The Model 8905 (see Figure 1-5) has internal reservoir positions, labeled to correspond to the reagent bottles, for:

- One wash solvent (A-train and B-train)
- Five ancillary reagents
- Five nucleotide monomer solutions (four for the standard nucleotides and one for special nucleotides or reagents).

Model 8905



Model 8909

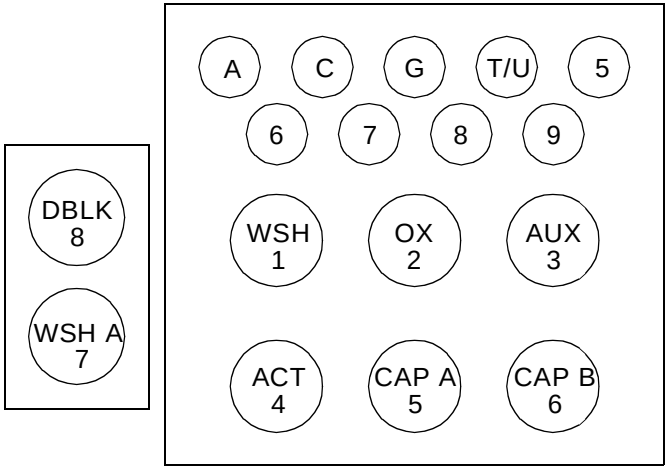


Figure 1-5 Reagent Reservoir Positions

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Réservoirs à Réactifs Modèle 8905

Dans l'Expedite 8905 (voir Figure 1-5), les emplacements des réservoirs à réactifs se trouvent tous à l'intérieur de l'appareil ; ils sont étiquetés et correspondent aux réactifs suivants :

- Un pour le solvant de lavage (circuit-A et circuit-B)
- Cinq pour les réactifs auxiliaires
- Cinq pour les solutions de monomères (quatre pour les solutions standard de nucléotides et une pour les nucléotides ou les réactifs spéciaux).

Model 8909 Reagent Reservoirs

The Model 8909 (see Figure 1-5) has internal reservoir positions, labeled to correspond to the reagent bottles, for:

- One wash solvent (B-train)
- Five ancillary reagents
- Nine nucleotide monomer solutions

Réservoirs à Réactifs Modèle 8909

Dans l'Expedite 8909 (voir Figure 1-5), une partie des réservoirs doit se placer à l'intérieur de l'appareil. Leurs emplacements sont étiquetés ; ils correspondent aux réactifs suivants :

- Un pour le solvant de lavage (circuit B)
- Cinq pour les réactifs auxiliaires
- Neuf pour les solutions de monomères

In addition, the Model 8909 has an external reagent tray that holds:

- Deblock solution
- External Wash solvent (A-train)

WARNING

Use only plastic-coated bottles in the external reagent tray.

NOTE: *All the bottles are pressurized at the same time. Therefore a bottle (even if it is empty) must be attached to each position before a synthesis is started.*

Les autres réservoirs se placent sur le support extérieur à savoir:

- La solution de détritylation ("Deblock")
- Le solvant de lavage externe (circuit A)

AVERTISSEMENT

Utilisez seulement les flacons revêtus de plastique sur le plateau extèrieur de rèactif.

Tous ces flacons sont mis sous pression en même temps. Par conséquent, un flacon (même vide) doit être positionné sur chaque emplacement avant le démarrage de la synthèse.

Reagent Kits

The Model 8905 Reagent Kit contains enough ancillary reagents for approximately 150 coupling cycles at the 0.2 μmole scale.

The Model 8909 Reagent Kit contains enough ancillary reagents for approximately 800 coupling cycles at the 0.2 μmole scale.

The amidites can be obtained in 0.25 g, 0.5 g, and 1.0 g amounts which is enough for approximately 150, 400, or 800 coupling cycles respectively.

Each reagent line has a 20 micron filter that prevents particles from entering the fluidic passages.

Reaction columns

Disposable columns prepacked with a controlled pore glass (CPG) with the 3'-nucleoside attached are available for the following:

- 0.05 μmole DNA synthesis
- 0.2 μmole DNA synthesis
- 1 μmole DNA synthesis
- 15 μmole DNA synthesis
- 1 μmole RNA synthesis

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CPG columns do not have a specific orientation so they can be connected in either direction.

Columns are packed with CPG with pore sizes of either 1000 Å or 500 Å. Columns with1000 Å CPG are recommended for long oligomers (≥50 mers) and columns with 500 Å CPG are recommended for shorter sequences.

Reaction devices with membrane solid-support

Disposable MemSyn™ devices containing a membrane solid-support with the 3'-nucleoside attached are available for the following:

- 0.05 μmole DNA synthesis
- 0.2 μmole DNA synthesis

Place MemSyn devices on the synthesizer with the printed side of the label facing up.

MemSyn devices are suitable for synthesis of long and short oligomers.

Waste System

Two waste reservoirs are located beside the instrument (Figure 1-6). Four waste lines (two for each column) exit the instrument cabinet directly behind the reaction columns (at the right rear). ORG1 and ORG2 waste lines are connected to the organic waste bottle cap. TRT1 and TRT2 waste lines are connected to the chlorinated waste bottle cap.

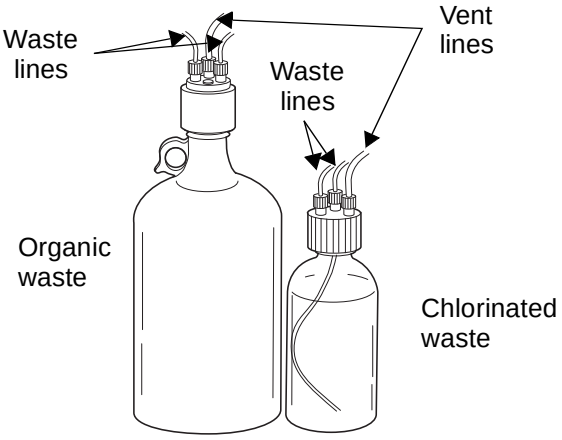


Figure 1-6 Waste Bottle and Connections

Keep waste reservoirs in the spill tray provided.

Drip Trays

The instrument contains three drip trays:

- Trityl monitor drip tray (inside the instrument cabinet)
- Column drip tray (not removable)
- Instrument cabinet drip tray (removable)

The trityl monitor drains into the column drip tray which then drains into the instrument cabinet drip tray. The instrument cabinet drip tray is removed easily (see Figure 1-4) by sliding it out of the instrument cabinet. In the event of a spill, the cabinet drip tray can hold the contents of the largest reagent bottle (1800 mL) without overflowing.

In the event of a leak all fluids are routed to the drip tray and flow toward the front of the instrument.

NOTE: *If reagents accumulate in the drip tray or the waste spill tray, use a spill pillow to absorb any liquid and dispose of the pillow safely (see* Appendix B, Reagent Safety*).*

Tiroirs d'Écoulement

L'appareil possède trois tiroirs d'écoulement :

- Un tiroir d'écoulement du détecteur de trityl (à l'intérieur du cabinet)
- Un tiroir d'écoulement des colonnes (inamovible)
- Un tiroir d'écoulement du cabinet de l'appareil (amovible).

Le tiroir d'écoulement du détecteur de trityl s'écoule dans le tiroir d'écoulement des colonnes, qui lui-même s'écoule dans le tiroir d'écoulement du cabinet. Celui-ci se retire facilement en le faisant glisser hors du cabinet (voir Figure 1-4). En cas de fuite, le tiroir d'écoulement du cabinet peut recevoir le contenu du plus grand flacon de réactif (1800 ml) sans déborder.

En cas de fuite, tous les fluides sont dirigés vers le tiroir d'écoulement et s'écoulent vers l'avant de l'appareil.

1

Si des réactifs s'écoulent dans le tiroir d'écoulement de l'appareil ou dans celui des rejets, épongez-les et jetez l'éponge selon les consignes de sécurité (voir "Appendix B, Reagent Safety", de ce manuel).

1.3.3 Pneumatic Control System

The pneumatic control system branches into high and low pressure systems within the cabinet:

- **Low pressure system** (6–7 psi, adjusted through an internal pressure regulator during installation)—Blankets the reagent bottles.
- **High pressure system** (20 psi, equal to inlet pressure)—Controls the pumping of reagents through the injectors by cycling gas pressure on a diaphragm.

Gas Supply

Use a dry helium gas supply for best performance. Dry helium is less soluble in the reagents and ensures a longer life for the chemicals, especially the monomers.

Connect a 2-stage regulator to the helium gas supply and adjust to 20 psi (±5 psi).

***NOTE**: If helium is not available, you can use argon or nitrogen (high purity, 99.995%). However, bubbles introduced by these gases may cause trityl failures.*

Relief Valves

The system includes two relief valves:

- **High pressure relief valve**—Prevents inlet gas from overpressuring the fluidic injectors. This relief valve is activated at 30 psi (±5 psi).

***NOTE**: Do not set inlet pressure above 20 psi. Higher inlet pressures can exceed the high pressure relief valve limit, and result in your gas supply bleeding through the relief valve.*

- **Low pressure relief valve**—Prevents the low-pressure blanket gas from over-pressurizing the reservoirs. If the pressure rises above the maximum of 15 psi, the relief valve automatically opens to reduce the pressure.

Gas Pressure Sensors

The instrument is equipped with two gas pressure sensor switches that monitor the gas pressure entering the instrument (high pressure sensor) and the blanket gas pressure (low pressure sensor). These alarms are activated by default in the User Profile (see Section 3.6.6, Specifying a User Profile).

If the high pressure falls below a specified level (10 psi), the “High pressure system failure” message is displayed on the screen and recorded in the Instrument Log. If the blanket pressure drops below 4.0 psi, the “Low pressure system failure” message is displayed on the screen and recorded in the Instrument Log. This indicates that one or more bottles are leaking, or there is a problem in the low pressure regulator.

If you acknowledge these messages immediately, the synthesis continues without interruption. If you fail to acknowledge the messages, the synthesis is halted at the end of the current cycle.

Gas Leak Detection

Diagnostics tools are available for the diagnosis and isolation of leaks. The Diagnostics tools, which are accessed through the Tools menu, provide routines for independent leak testing of the High and Low pressure systems.

Gas Saver

The Gas Saver reduces overall gas consumption and automatically detects significant leaks. The Gas Saver is activated by default in the User Profile (see Section 3.6.6, Specifying a User Profile).

1

If the instrument has been idle for 5 minutes (no keyboard or pumping activity), the Gas Saver is activated and remains operational until the instrument is activated (a key is pressed). The Gas Saver shuts off the gas until the blanketing pressure falls below 4.0 psi when the pressure is restored. During periods of inactivity, the system periodically performs the leak test and shuts off the gas supply if a failure is detected.

1.3.4 Power Control System

The power control system, which is isolated at the rear of the instrument, accepts AC power and supplies DC regulated power to the microprocessor and the pneumatics system. The power entry cord and fuses are contained in this module (see Figure 1-7).

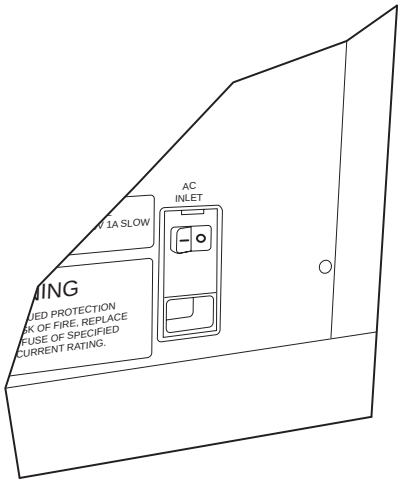


Figure 1-7 Power Entry and Fuses

The power supply is shielded from reagent leaks by appropriate placement and isolation of electronic assemblies and instrument cables.

No voltage selection is required. The Expedite Nucleic Acid Synthesis System is equipped with a universal power supply that can be used for 100 V or 240 V operation. The use of a surge suppressor between the instrument and the electrical outlet is recommended.

1.4 Safety Precautions

The following information is provided to ensure safe operation of the Expedite Nucleic Acid Synthesis System. Please read it carefully before using the instrument and observe the following safety recommendations.

Les informations suivantes vous sont communiquées afin de garantir votre sécurité lors de l'utilisation du synthétiseur d'acides nucléiques Expedite. Lisez-les attentivement avant de l'utiliser et respectez les consignes de sécurité.

Electrical

Two shielded AC cords are provided. Select and use the cord that is compatible with your main power supply.

Unplug the main power cable before you service or repair the instrument.

WARNING

To prevent electric shock, do not remove the instrument cover. There are no user serviceable parts within the cabinet. Refer servicing to qualified personnel.

Electricité

Utilisez uniquement le fil électrique protégé fourni avec l'appareil. Débranchez impérativement le câble d'alimentation principal avant de procéder aux opérations d'entretien ou de réparation de l'appareil.

1

AVERTISSEMENT

Afin d'éviter les chocs électriques, ne retirez pas le couvercle de l'appareil. Il n'y a à l'intérieur de l'appareil aucune pièce que vous puissiez remplacer vous-même. Faites appel au service technique de PerSeptive Biosystems.

Gas Supply

Securely anchor the tank of pressurized gas.

Use a 2-stage regulator to step the pressure down to 20 psi.

***NOTE**: Do not set inlet pressure above 20 psi (±5 psi). Higher inlet pressures can exceed the high pressure relief valve limit, and result in your gas supply bleeding through the relief valve.*

Gaz

Fixez solidement le réservoir de gaz sous pression.

Nous recommandons l'utilisation d'un régulateur de pression à deux étages permettant d'obtenir une pression de sortie de 20 psi (1,3 bar).

Ne pas régler la pression d'entrée au dessus de 20 psi (1,3 bar) ±5 psi (0,3 bar). Des pressions d'entrée plus élevées peuvent dépasser la limite supérieure de pression du détendeur, et causer la fuite de l'alimentation de gaz dans le détendeur.

Operating Temperature

The protocols are optimized for room temperature operation (20 to 30 ^{o}C).

Température de Fonctionnement

Les protocoles sont optimisés pour un fonctionnement à température ambiante (20 à 30 $^{\circ}$C).

Vent the Instrument

The instrument cabinet is equipped with an exhaust fan that vents vapors to the rear of the instrument. Make sure that the vent tube on the back of the instrument is connected to a suitable exhaust duct.

Ventilation de l'appareil

L'appareil est équipé d'un ventilateur qui évacue les vapeurs vers l'arrière de l'appareil. Assurez-vous que le tube évent à l'arrière de l'appareil est bien raccordé à un conduit d'échappement.

Reagent Handling

Most of the reagents and solvents used in nucleic acid synthesis are hazardous. Wear a lab coat, gloves and eye protection when handling reagents. Adequate ventilation is essential and working under a fume hood is recommended.

Flammable reagents should be kept in an appropriate flameproof cabinet. Oxidizers should be isolated. Consult Appendix B, Reagent Safety, for information on the handling of specific chemicals.

Make sure that you put the reagent bottles on the instrument in the correct position. Mixing up the bottles will contaminate the instrument. The reagent bottles on the instrument are pressurized. Take care when removing the bottles from the instrument.

Manipulation des Réactifs

La plupart des réactifs et des solvants employés en synthèse d'acides nucléiques sont dangereux. Portez une blouse, des gants et des lunettes de protection lorsque vous les manipulez. Une ventilation adéquate est nécessaire et il est recommandé de travailler sous une hotte.

Les réactifs inflammables doivent être stockés dans des armoires métalliques. Les oxydants doivent être stockés séparément. Reportez-vous à la partie “Appendix B, Reagent Safety”, de ce manuel, pour de plus amples informations sur les manipulations de produits chimiques particuliers.

Faites en sorte de positionner les flacons de réactifs à leur place dans l'appareil. Intervertir les bouteilles contaminerait l'appareil. Les bouteilles de réactifs sont sous pression lorsque l'appareil fonctionne. Prenez garde lorsque vous les retirez de l'appareil.

1

Waste Disposal

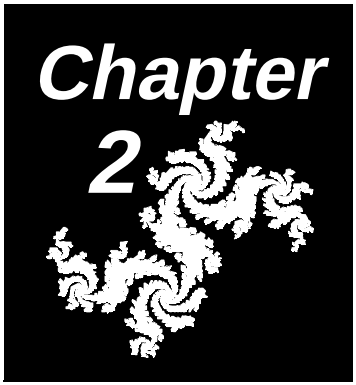
Collect the waste generated from DNA synthesis and dispose of it in accordance with local, state, and federal regulations pertaining to toxic waste removal.

Elimination des Solvants et Réactifs Usagés

Récupérez les rejets issus de la synthèse d'ADN et détruisez-les en suivant les directives en vigueur sur l'élimination des déchets toxiques.

Boot diskette removal

Do not remove the boot diskette from the disk drive when the system is powered up. The system continuously accesses the boot diskette. Power down the system before removing the diskette to avoid damaging the diskette or diskette drive.



2 Performing a Synthesis

This chapter contains the following sections:

2.1 Introduction

This chapter describes the step-by-step procedure for performing a synthesis on the Expedite Nucleic Acid Synthesis System. You can perform the following chemistries on this instrument:

- β-Cyanoethyl Phosphoramidite DNA Synthesis
- β-Cyanoethyl Phosphoramidite DNA Synthesis with Expedite™ Monomers
- β-Cyanoethyl Phosphoramidite RNA Synthesis
- β-Cyanoethyl Phosphoramidite RNA Synthesis with Expedite™ Monomers
- Phosphorothioated DNA synthesis

In this tutorial, operation of the software is discussed as needed. A detailed description of the software is provided in Chapter 3, Software Reference.

Key Steps in a Synthesis

The key steps to perform a synthesis are:

1. Turn on the instrument.
2. Specify the chemistry to be used.
3. Create the sequence.
4. Install or replenish the reagents.
5. Prime the fluidic system.
6. Install the reaction column(s).
7. Perform the Synthesis.
8. Cleave the product from the reactor support.
9. Perform post-synthesis base deprotection of the product.
10. Optionally, analyze and purify the product.

2.1.1 Beta-Cyanoethyl Phosphoramidite Synthesis

The patented β-cyanoethyl phosphoramidite chemistry is the method of choice in DNA synthesis. This method provides:

- High coupling efficiency
- Reduced side reactions which maintain high biological activity

Each cycle (see Figure 2-1) is composed of the following steps with intervening wash steps to remove excess reactants and reaction by-products:

- Deblocking
- Coupling
- Capping
- Oxidation
- Capping

Following the final capping step, the cycle of reactions is repeated again, beginning with deblocking, until chain elongation is complete. The oligomer is then cleaved from the support and deprotected. If desired you may then purify the product.

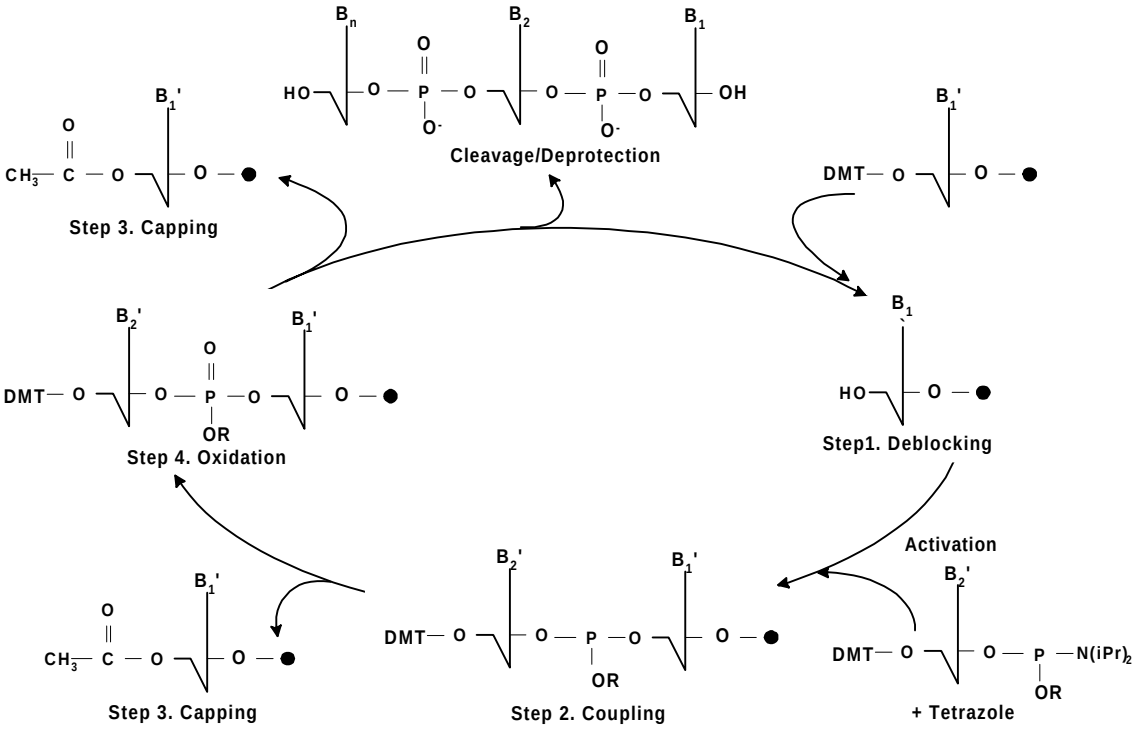


Figure 2-1 Phosphoramidite Synthesis Cycle

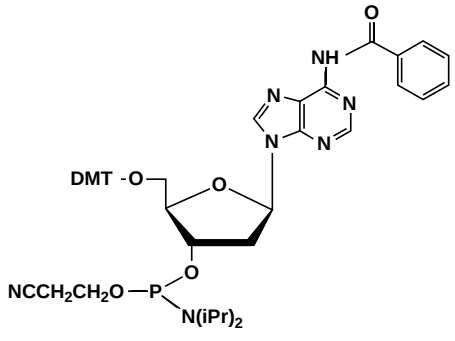
Phosphoramidite Monomers

The phosphoramidite monomers, shown in Figure 2-2, are protected as follows:

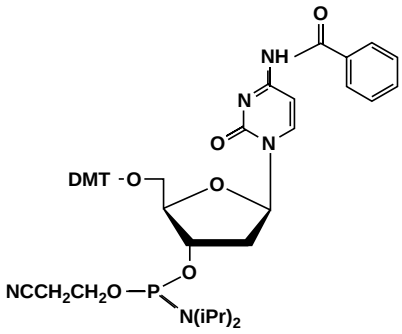
- The 5'-hydroxyl positions are protected with DMT (dimethoxytrityl) groups.
- Phosphorus is protected with diisopropyl amine groups and a β-cyanoethyl group.

The exocyclic amines are protected as follows:

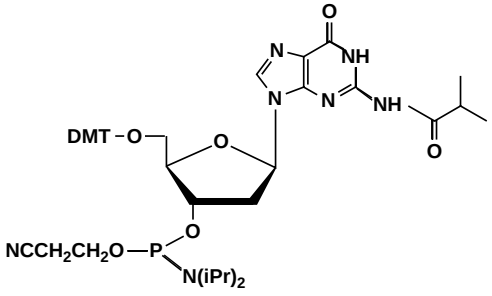
- Adenosine and cytidine are protected with benzoyl groups (Bz).
- Guanosine is protected with isobutyryl (iBu).
- Thymidine does not need to be protected.



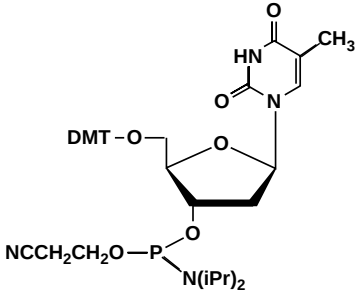
5'-DMT-dAdenosine (N^6-Benzoyl) β-Cyanoethyl Phosphoramidite



5'-DMT-dCytidine (N^4-Benzoyl) β-Cyanoethyl Phosphoramidite



5'-DMT-dGuanosine (N^2-Isobutyryl) β-Cyanoethyl Phosphoramidite



5'-DMT-Thymidine β-Cyanoethyl Phosphoramidite

Figure 2-2 Phosphoramidite Monomers

2.1.2 DNA Synthesis with Expedite Monomers

The β-Cyanoethyl Phosphoramidite synthesis of DNA oligomers using Expedite monomers[1]:

- Decreases deprotection time
- Enables milder deprotection conditions
- Is compatible with multiple synthesis chemistries

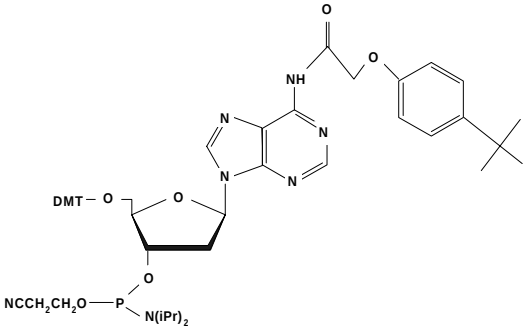
You can directly substitute Expedite monomers for standard monomers without changing the synthesis protocols. All solutions are the same except for the Cap A reagent which is tert-butylphenoxyacetic anhydride in THF.

Expedite Monomers

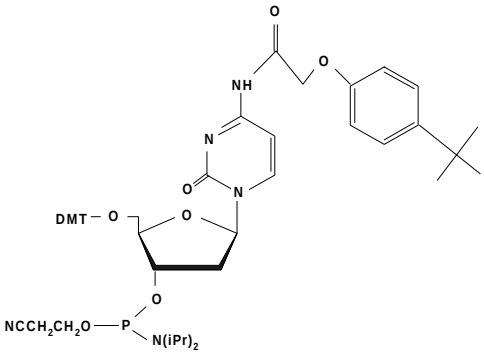
The Expedite phosphoramidite monomers (see Figure 2-3) are protected as follows:

- The 5'-hydroxyl positions are protected with DMT groups.
- Phosphorus is protected with diisopropyl amine groups and a β-cyanoethyl group.
- The exocyclic amines of adenosine, guanosine and cytidine are protected with a new, stable base-protecting group, tert-butylphenoxyacetyl (tBPA).
- Thymidine does not need to be protected.

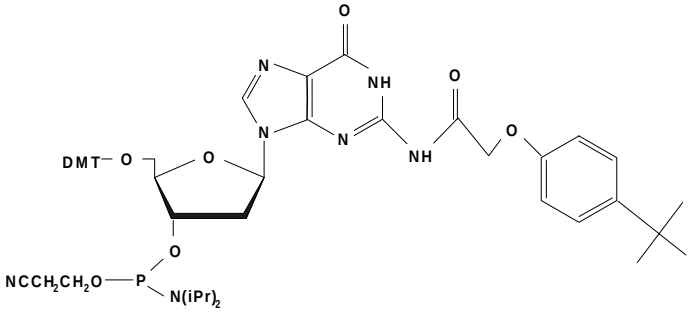
1. Sinha, N. D., Davis, P., Usman, N., Perez, J., Hodge, R., Kremsky, J. and Casale, R. (1993) Biochimie Vol. 75 p 13-23. “Labile exocyclic amine protection of nucleosides in DNA, RNA, and oligonucleotide analog synthesis facilitating N-deacylation, minimizing depurination and chain degradation.”



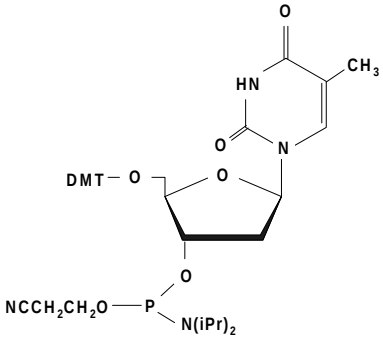
5'-DMT-dAdenosine (N^6-tBPA)
β-Cyanoethyl Phosphoramidite



5'-DMT-dCytidine (N^4-tBPA
β-Cyanoethyl Phosphoramidite



5'-DMT-dGuanosine (N^2-tBPA)
β-Cyanoethyl Phosphoramidite



5'-DMT-Thymidine
β-Cyanoethyl Phosphoramidite

Figure 2-3 Expedite Monomers

2.1.3 RNA Synthesis

The RNA synthesis cycle is the same as the β-cyanoethyl phosphoramidite DNA synthesis cycle (see Figure 2-1) except that RNA synthesis uses different nucleoside monomers and longer coupling times are required. The same ancillary reagents are used and the sequence of the synthesis steps is the same, so it is easy to switch between RNA and DNA synthesis.

Standard RNA Monomers

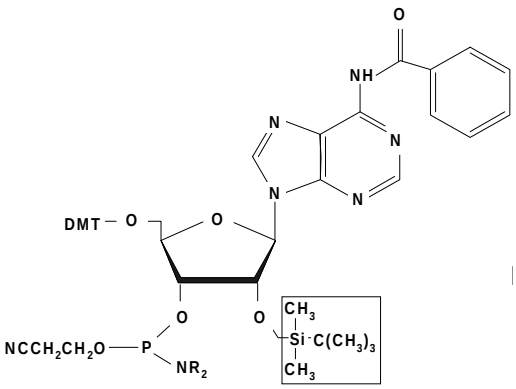
The difference between the β-cyanoethyl phosphoramidite monomers that are used in RNA synthesis and those that are used in DNA synthesis is the presence of an additional protecting group at the 2'-hydroxyl position of the ribose ring.

The RNA phosphoramidite monomers, shown in Figure 2-4, are protected as follows:

- The 5'-hydroxyl positions are protected with DMT groups.
- The 2'-hydroxyl positions are protected with t-butyldimethylsilyl (t-BDMS) groups. The t-BDMS groups are stable in the acidic conditions used to remove the DMT group and are readily removed after cleavage/deprotection to produce natural RNA.
- Phosphorus is protected with diisopropyl amine groups and a β-cyanoethyl group.

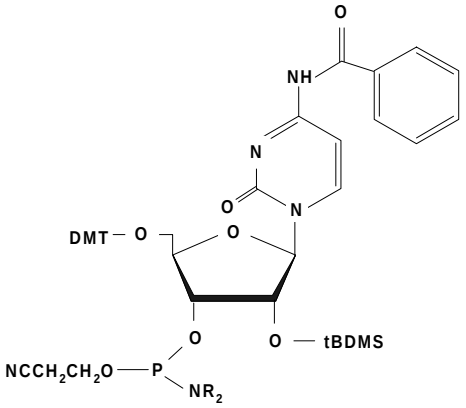
The exocyclic amines are protected as follows.

- Adenosine and cytidine are protected with benzoyl groups (Bz).
- Guanosine is protected with isobutyryl (iBu).
- Thymidine is replaced with Uridine which does not need to be protected.

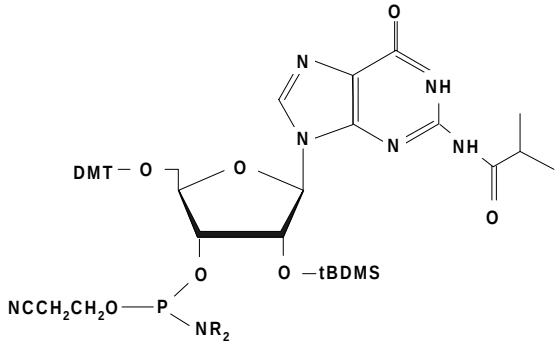


5'-DMT-Adenosine (Benzoyl)
β-Cyanoethyl Phosphoramidite

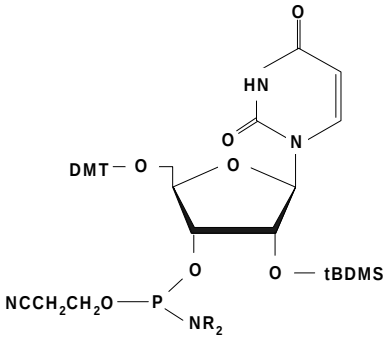
(t-BDMS



5'-DMT-Cytidine (Benzoyl)
β-Cyanoethyl Phosphoramidite



5'-DMT-Guanosine (Isobutyryl)
β-Cyanoethyl Phosphoramidite



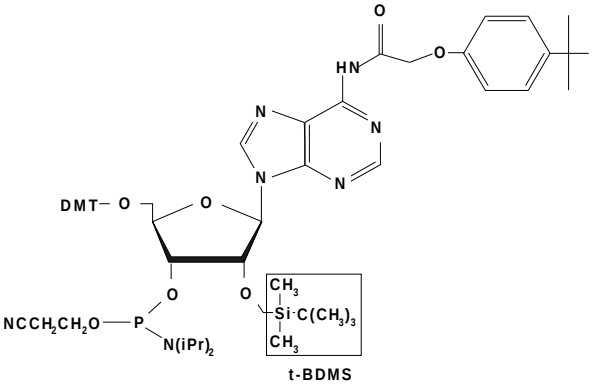
5'-DMT-Uridine
β-Cyanoethyl Phosphoramidite

Figure 2-4 Standard RNA Monomers

Expedite RNA Monomers

Expedite RNA phosphoramidite monomers use a new, stable exocyclic amine protecting group *tert*-butylphenoxyacetyl (*t*BPA) that dramatically decreases deprotection time. The shorter exposure time to ammonia reduces the hydrolysis of the silyl groups resulting in less chain degradation.

Expedite Chemistry	Standard RNA Chemistry
Heat the sealed tube at 55°C for 15 minutes or Let the reaction proceed at room temperature for 2 hours.	Heat the sealed tube at 55°C for 6 to 16 minutes or Let the reaction proceed at room temperature for 24 hours.



5'-DMT-Adenosine (Tert-butylphenoxyacetyl)
β-Cyanoethyl Phosphoramidite

Figure 2-5 Structure of an Expedite RNA Monomer

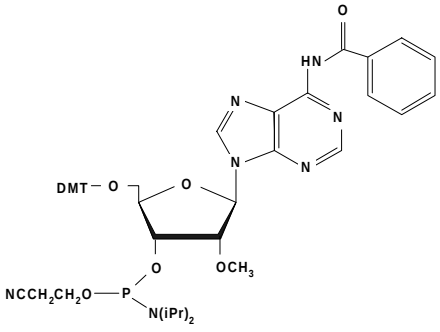
2'-O-Methyl RNA Monomers

Research requiring RNA has increased dramatically, resulting in greater need for oligonucleotides. In research where specific RNA linkages are not required, there are considerable practical benefits for the use of 2'-O-Methyl phosphoramidite monomers:

- Ease of handling that is more like that of DNA and RNA
- Higher coupling efficiency
- Greater nuclease resistance
- Use in applications that require the higher binding of RNA-RNA relative to DNA-DNA

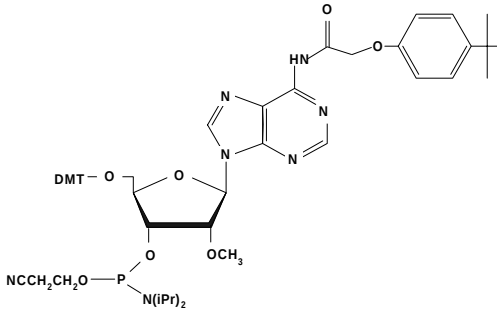
2'-O-Methyl phosphoramidite monomers are available in standard and Expedite chemistries.

Standard



N^6-Benzoyl-5'-O-DMT-
2'-O-Methyl-Adenosine-3'-
(β-Cyanoethyl) Phosphoramidite

Expedite



N^6-Tert-butylphenoxyacetyl-5'-O-DMT-
2'-O-Methyl-Adenosine-3'-
(β-Cyanoethyl) Phosphoramidite

Figure 2-6 Structures for 2'-O-Methyl Phosphoramidite A Monomers

2.1.4 Phosphorothioated DNA Synthesis

The Expedite Nucleic Acid Synthesis System also provides protocols that allow you to synthesize DNA oligomers containing sulfur. These protocols use β-cyanoethyl phosphoramidite chemistry and a sulfurizing reagent such as 3 H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent) instead of the normal oxidizing reagent. The mechanism for the reaction is shown in Figure 2-7.

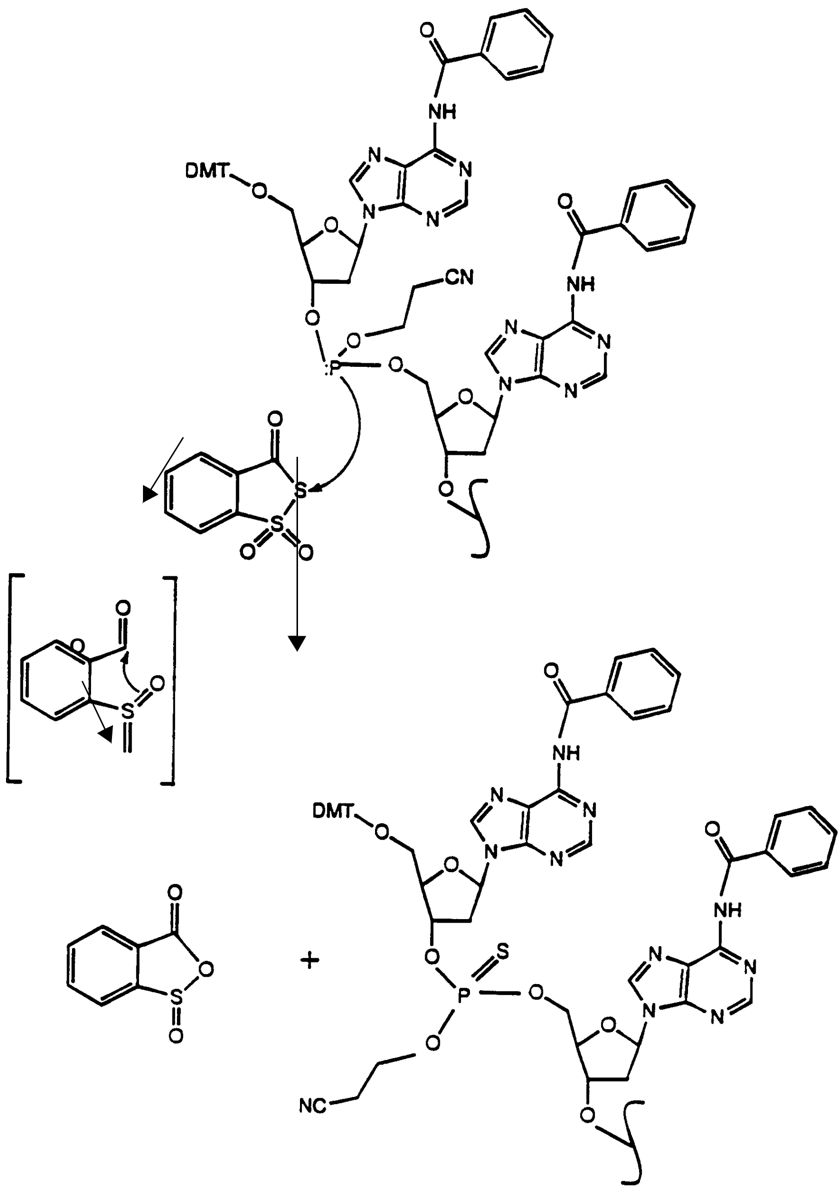


Figure 2-7 Sulfurizing Mechanism

Silanized reservoir requirement

Use a silanized reservoir for the sulfurizing agent to ensure reagent stability.

You can purchase silanized bottles (from Glen Research, for example), or you can silanize the reservoir yourself.

***NOTE**: On a 8905 system, the sulfurizing agent is placed in the Oxidizer bottle. Thoroughly clean the Oxidizer bottle, cap, and lines before silanizing the bottle and installing the sulfurizing agent. Oxidizing agent will cause the sulfurizing agent to precipitate.*

WARNING

The reagents and solvents used in this procedure are hazardous. Wear a lab coat, gloves and eye protection. Adequate ventilation is essential and working under a fume hood is recommended.

AVERTISSEMENT

Les réactifs et solvants employés dans cette procédure sont dangereux. Portez une blouse, des gants et des lunettes de protection. Une ventilation adéquate est indispensable et il est recommandé de travailler sous une hotte.

To clean the vessel with concentrated sulfuric acid and silanize it:

1. Immerse the vessel in concentrated sulfuric acid for approximately 15 hours.
2. Wash the vessel with water and dry it in an oven to remove all moisture.

3. Immerse the vessel in a 10% solution of dichlorodimethylsilane (DCMS) in dichloromethane (DCM) for 3 to 5 minutes.
4. Rinse the vessel thoroughly with methanol to quench the reaction.
5. Place the vessel in a 100 ^{o}C oven for 1 hour to dry out the methanol.
6. Cool the reservoir then fill it with the sulfurizing reagent (see Section 2.4.2, Installing the Reagents).

2.1.5 Selecting the Chemistry

The default chemistry used on the Expedite Nucleic Acid Synthesis System is the β-cyanoethyl phosphoramidite DNA method. This chemistry is specified in the standard User Profile.

You can change the type of chemistry that the system performs in two ways:

- Change the chemistry in the User Profile. See Section 3.6.7, Changing the Chemistry, to modify the User Profile.
- Change the case of the base designators in the sequence:

Chemistry	Uppercase	Lowercase
DNA	DNA	Thio
Thio	Thio	DNA
RNA	RNA	DNA

2.2 Powering Up the System

To power up the instrument:

1. Make sure that there is a bottle firmly attached to each reservoir position (see Figure 2-8).

   The bottles should be “finger tight.” Do not overtighten the bottles because this deforms the sealing O-rings and may cause gas leakage.

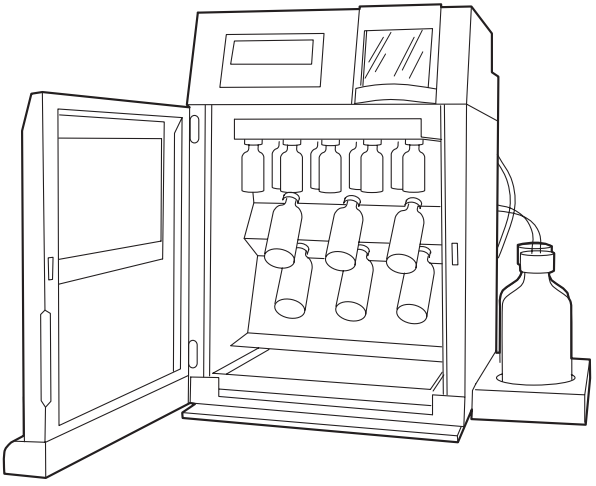


Figure 2-8 Install Bottles Before Powering up the Instrument

2. Turn the instrument on (the on/off switch is located at the left rear of the unit, Figure 2-9).

   If the boot diskette is not inserted in the drive, you are prompted to do so. Turn off the power and insert the diskette in the drive (see Figure 2-9). Turn the instrument on.

NOTE: *Remove the diskette only to install software upgrades. Power down before removing the diskette. Insert the new diskette before powering up.*

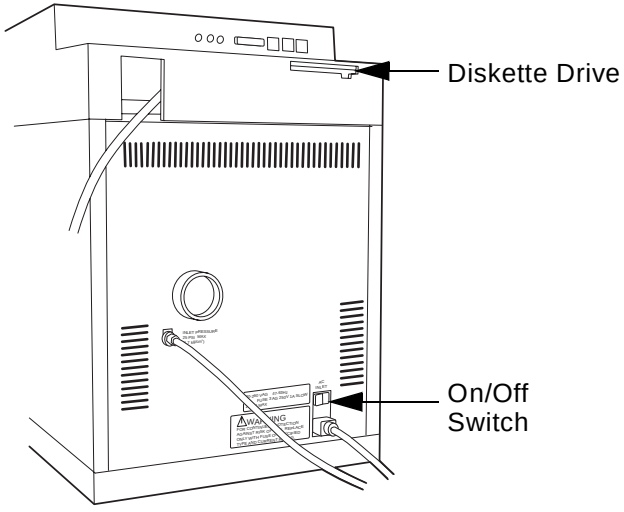


Figure 2-9 Instrument Cabinet Showing On/Off Switch

The software is loaded and the system is initialized. The instrument then runs a series of self-diagnostic routines. During the initialization procedure, the screen shown in Figure 2-10 is displayed.

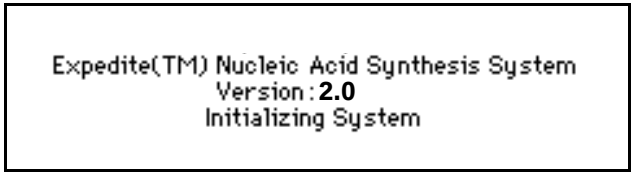


Figure 2-10 System Initialization Display

2

CAUTION

The boot diskette contains instrument operating instructions and the current operational parameters. Do not remove the diskette while the instrument is operating. Power down before removing the diskette to avoid damaging diskette or diskette drive.

When the instrument passes all diagnostics, the Main menu, shown in Figure 2-11, is displayed.

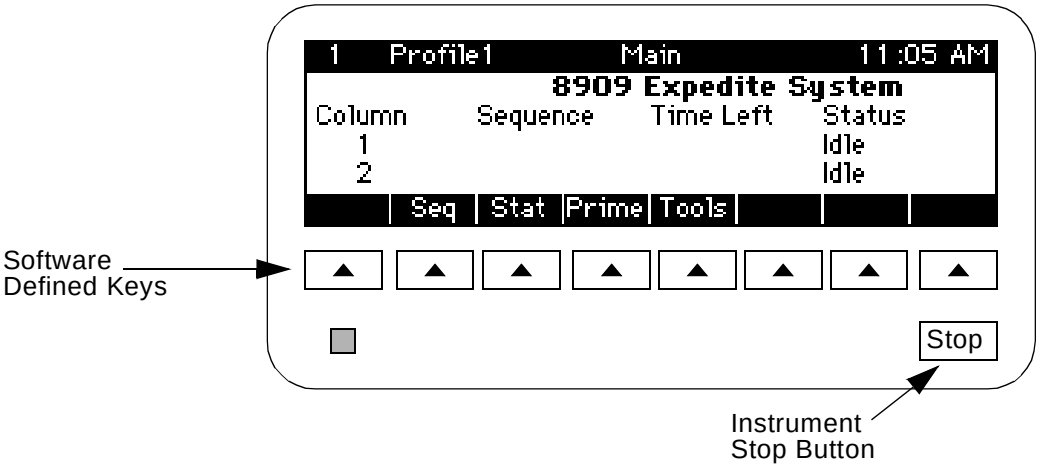


Figure 2-11 Main Menu and Soft Keys

If the instrument is unable to complete the diagnostic routines a continuous beep will sound and the Main menu may not appear.

Selecting Menu Options

Different functions are assigned to the soft keys in each screen. The key assignment is displayed at the bottom of each screen. To select a menu option, press the soft key directly below the desired option (see Figure 2-11).

As a rule the furthest soft key to the right (eighth) is an **Exit** key that is used to go back to the previous menu screen.

Some menus have more than eight options. In this event, the seventh soft key is labeled **More**. When you select this key, an additional screen, in which new functions are assigned to the soft keys, is displayed.

In operations that can be aborted, the seventh soft key is labeled **cancel**. Select this key to cancel the operation.

Pressurizing the Instrument

The instrument is automatically pressurized when you:

- Power up the system with gas attached at 20 psi.
- Press any key on an idle instrument.

NOTE: *All bottles are pressurized together. You must attach an empty bottle to any unused instrument position.*

The Expedite Nucleic Acid Synthesis System is equipped with high and low gas pressure sensors.

The high pressure sensor detects gas entering the system from the external tank and regulator. If the high pressure sensor fails to detect any pressure above 10 psi within 5 seconds, a warning is issued on the display. If the instrument is running a synthesis, the synthesis is halted at the end of the current cycle. The instrument remains halted until you acknowledge this warning and restart the synthesis. Triggering of this alarm is usually a signal that the gas tank is depleted and needs to be replaced.

The low pressure sensor, which has a threshold of 4 psi, monitors the reagent reservoirs. A failure of the low pressure system is usually an indication that a reagent reservoir is not sealed on the instrument.

The high and low pressure sensors and the Gas Saver are activated when you first start the instrument. Whenever the instrument is automatically pressurized, a gas leak test is performed (see Section 3.6.1, Diagnostic Routines). If the leak test fails, a warning is issued. You must acknowledge the warning before you can continue with a synthesis.

You can disable the gas sensors and the leak test by editing the User Profile (see Section 3.6.6, Specifying a User Profile). However, this is not recommended.

Failure to Pass Startup Leak Test

If the instrument fails the startup leak test, a warning is displayed on the screen (see Figure 2-12). You must acknowledge the warning by pressing **ok** before you can continue.

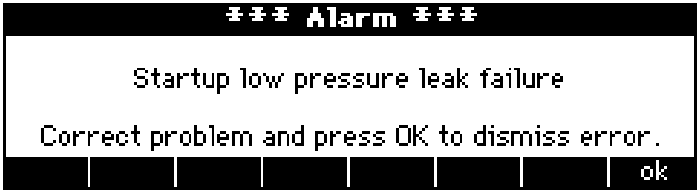


Figure 2-12 Leak Test Failure Warning

If you acknowledge the warning but do not locate the leak, the synthesis will continue but the warning will be repeated each time the leak test is performed. Perform the tests described in Section 4.2, Gas Leak Diagnostics, to locate the leak and take corrective action before continuing with the synthesis.

Gas Saver Leak Test

The gas saver leak test reduces overall gas consumption. The gas saver is activated when the instrument has been idle for 5 minutes (no keyboard or valve pumping activity).

During periods of inactivity, the Gas Saver periodically turns off both the high and low pressure inlet valves and measures the time taken for the blanketing pressure to fall below 4 psi. If this decay takes one minute or less, the instrument interprets this as a gross leak (for example, a bottle has been removed) and turns off the inlet valves until the instrument is activated and you acknowledge the gas saver test fail signal (Figure 2-13).

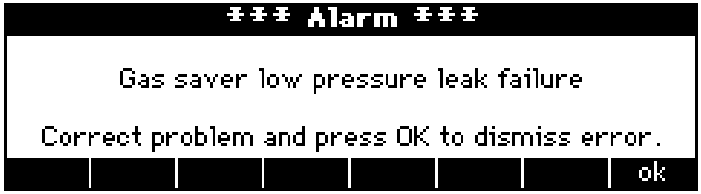


Figure 2-13 Gas Saver Leak Test Failure Message

If the decay period is greater than one minute, the Gas Saver shuts off the gas until the blanketing pressure falls below 4.0 psi when the pressure is restored. The instrument continues to repressurize and time the period of decay until the instrument is activated.

2.3 Entering the Sequence

Use the Sequence Editor to enter a new sequence or edit an existing sequence for synthesis:

NOTE: *The Sequence Editor is described in detail in* Section 3.3, Sequence Menu*.*

1. If the **Main** menu is not displayed, press **Exit** until the Main menu is displayed (see Figure 2-14).

Figure 2-14 Main Menu

2. From the Main menu select **Seq.**

   The Sequence menu, shown in Figure 2-15, is displayed.

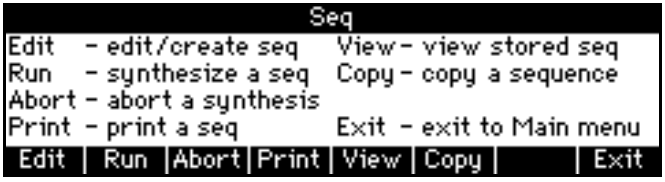


Figure 2-15 Sequence Menu

3. Select **Edit**.

The screen shown in Figure 2-16 is displayed.

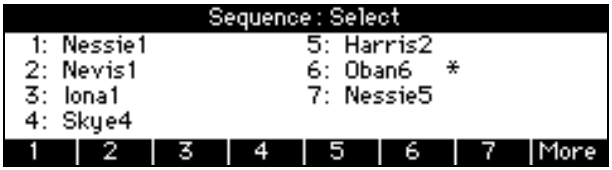


Figure 2-16 Sequence Selection Screen

4. Select the sequence (1 to 63) you wish to edit. Press the More key to display additional sequences.
   - If you select a new sequence, a new screen is displayed (see Figure 2-17) to enable you to enter the sequence.
   - If you select an existing sequence, the selected sequence is displayed.

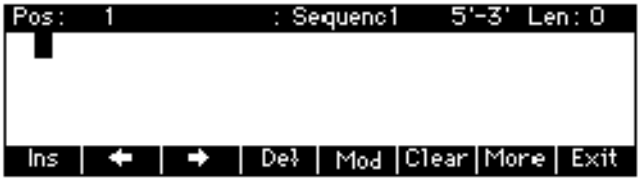


Figure 2-17 Sequence Screen

5. Select **Ins** (insert).
6. Enter your sequence by pressing the entry mode keys (see Figure 2-18). Refer to Table 3-4 for a description of all entry mode keys. For mixed site entry, see "Mixed Site Entry" on page 3-16.

NOTE: *If you are entering an RNA sequence, U (Uridine) will be displayed instead of T (thymidine).*

The sequence is displayed on the screen (see Figure 2-18).

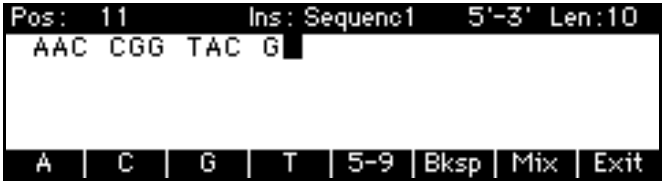


Figure 2-18 Sequence Edit Screen (Model 8909)

NOTE: *Take note of the 5' - 3' orientation of the displayed sequence, listed in the upper right of the display. You can change the display orientation by modifying the user profile (see Section 3.6.6, Specifying a User Profile).*

You can use the **Bksp** key to correct mistakes or select **Exit** to back up one menu and gain access to the cursor keys ← and →.

From this menu, you can move to any point in the sequence and insert or delete bases in the sequence. The procedures for entering sequences are described in detail in Section 3.3.1, Using the Sequence Editor.

You can also name your sequence by selecting **More** followed by **Name** (see "Naming a Sequence" on page 3-23).

7. When you have finished entering the sequence, select **Exit** repeatedly until the screen shown in Figure 2-19 is displayed.

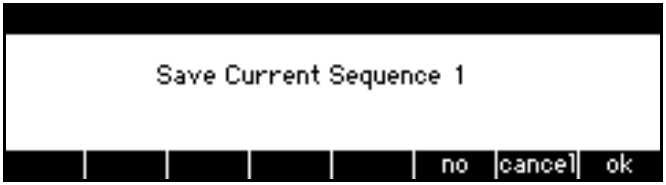


Figure 2-19 Save the Sequence

8. Press **ok** to save the sequence.
9. The Sequence menu is displayed. Press **Exit** until the Main menu is displayed.

2.4 Preparing and Loading the Reagents

WARNING

Most of the reagents and solvents used in nucleic acid synthesis are hazardous. Wear a lab coat, gloves and eye protection when handling reagents. Adequate ventilation is essential and working under a fume hood is recommended.

AVERTISSEMENT

La plupart des réactifs et solvants employés en synthèse d'acides nucléiques sont dangereux. Portez une blouse, des gants et des lunettes de protection lorsque vous manipulez des réactifs. Une ventilation adéquate est nécessaire et il est recommandé de travailler sous une hotte.

The reagents used in nucleic acid synthesis are extremely hygroscopic. As it is essential that the reagents remain anhydrous, work quickly to minimize the exposure to air. Bring the monomer bottles to room temperature before opening. This will minimize any moisture contamination when the bottles are opened.

CAUTION

Whenever you change chemistry, you must rinse out the fluidics module with acetonitrile. See Section 3.5.7, Shutdown.

2.4.1 Reagents

The reagents that are used with the chemical protocols on the Expedite Nucleic Acid Synthesis System are listed in Table 2-1.

Table 2-1 Reagents Used in Nucleic Acid Synthesis

Reagent	β-Cyanoethyl Phosphoramidite DNA Synthesis and RNA Synthesis	Expedite Monomers	Sulfur Containing DNA
WSH	Dry Acetonitrile (less than 0.005% H_2O)		
WSH A	Acetonitrile		
DBLK	Trichloroacetic acid (TCA) in dichloromethane (DCM)		
ACT	Tetrazole in acetonitrile		
CAP A	Acetic anhydride in pyridine/THF	t-Butylphenoxy acetic anhydride in THF	Acetic anhydride in pyridine/THF
CAP B	N-Methylimidazole in pyridine/THF		
OX	Iodine solution in THF/H_2O/pyridine		3 H-1,2-Benzodithiol-3-one 1,1-dioxide in Acetonitrile (8905)
AUX	Empty	Empty	3 H-1,2-Benzodithiol-3-one 1,1-dioxide in Acetonitrile (8909)

2.4.2 Installing the Reagents

Before you unscrew a bottle, select **Tools, Bottle** and select the bottle. Doing so sets the Automatic Deblanket gas to a state appropriate for the selected bottle:

- **Amidites, Activator and Wash (B-train) reagents**—Blanketing gas pressure is retained to ensure an inert atmosphere near the open reagent position and to flush the new reagent bottle with inert gas as it is installed.

  When you remove the reagent bottles on which the blanketing gas pressure is retained, the blanket gas escapes and you hear a hissing noise.

- **A-train reagents (Oxidizer, Deblock, Aux, Wsh A, Cap A and Cap B)**—Blanketing gas pressure is suspended to minimize odors. During blanket suspension, the Gas Saver and all pressure alarms are disabled.

WARNING

When you remove a bottle on which the blanketing gas pressure is retained, the blanket pressure in the bottle will cause reagent vapor to escape into the atmosphere. The exhaust fan within the instrument directs this vapor out the rear of the cabinet.

Certain reagents emit a noxious odor that cannot be exhausted fast enough by the fan. Make sure that the exhaust duct in the rear of the instrument is connected to a fume hood to vent these odors.

AVERTISSEMENT

Lorsque vous retirez un flacon de l'appareil, la pression résiduelle entraîne l'émission de vapeurs de réactifs. Le ventilateur placé à l'intérieur de l'appareil dirige cette vapeur vers l'arrière du cabinet.

Certains réactifs émettent une odeur nauséabonde qui n'est pas éliminée suffisamment rapidement par le ventilateur. Assurez-vous que le conduit d'échappement à l'arrière de l'appareil est bien connecté à une hotte pour éliminer ces odeurs.

Install one reagent at a time. Replace the bottles on the instrument as soon as possible. If a reagent bottle is not installed in an open position within a few minutes, the system will assume that the bottle change routine has been interrupted and will discontinue reagent blanketing.

Automatic Deblanket

The default mode for the Bottle Change Tool is for the Automatic Deblanket to be turned on. By default, when the Bottle Change Tool is selected, the blanketing gas pressure is retained while the Amidites, Activator and Wash (B-train) reagents, which must be kept moisture free, are being installed. To minimize odors, the blanket gas is automatically suspended while the A-train reagents (Oxidizer, Deblock, Cap A and Cap B) are being installed. During blanket suspension, the Gas Saver and all pressure alarms are disabled.

If desired, you can disable this automatic shutoff by turning off the Automatic Deblanket option in the User Profile (see Section 3.6.6, Specifying a User Profile).

Loading the Reagents

Select the appropriate reagent kit for the chemistry to be performed and unpack the reagents and place them in the vicinity of the instrument. Make sure the appropriate Reagent Kit and Chemistry are selected in the User Profile (see Section 3.6.6, Specifying a User Profile).

The contents of the Reagent Kits for the Models 8905 and 8909 are listed in Table 2-1. The positions of the reagent reservoirs in the Models 8905 and 8909 are shown in Figure 2-20.

Table 2-1 Expedite Reagent Kits

Reagent	Position #		Model 8905	Model 8909
	8905	8909		
Wash (WSH)	1		450 mL	300 mL
Wash (WSH A)	N/A	7	N/A	2000 mL
Oxidizer (OX)	2		60 mL	200 mL
Deblock (DBLK)	3	8	180 mL	900 mL
Activator (ACT)	4		60 mL	200 mL
Cap A (CAP A)	5		60 mL	200 mL
Cap B (CAP B)	6		60 mL	200 mL
Amidite Vials	A—5	A—9	0.25 mg (150 cycles)	0.5 g (400 cycles) 1.0 g (800 cycles)
Amidite Diluent (septum sealed)	NA		50 mL	100 mL

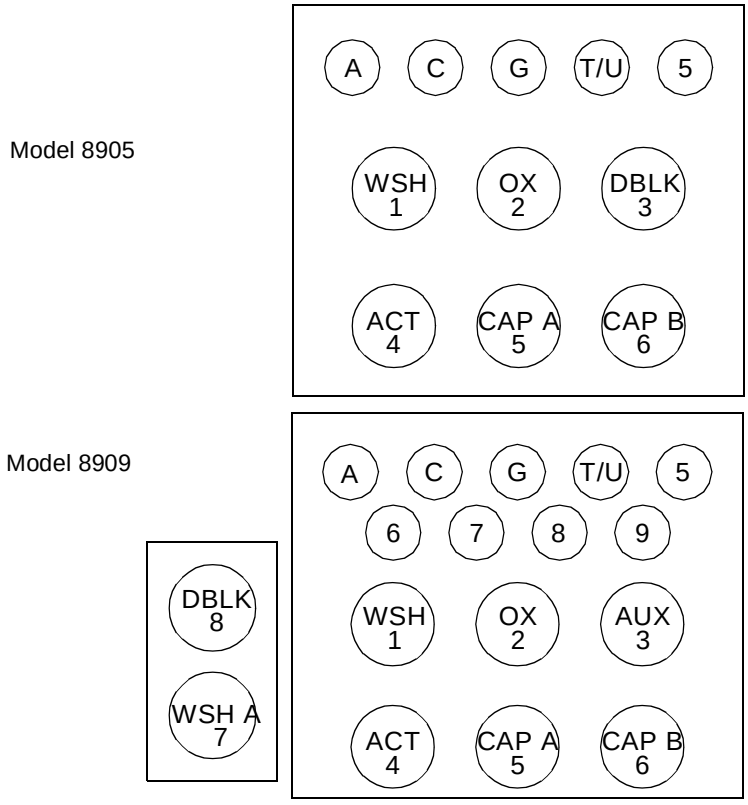


Figure 2-20 Models 8905 and 8909 Reagent Reservoir Positions

Install one reagent at a time in the following order:

1. Wash A (WSH A—Model 8909 only)
2. Deblock (DBLK)
3. Oxidizer (OX)
4. Capping reagents (CAP A, CAP B)
5. Wash reagent (WSH)
6. Activator (ACT)
7. Amidites

Model 8909 Install Wash A Reagent

To install the Wash A reagent on the Model 8909:

1. From the Main Menu select **Tools**.

   The screen shown in Figure 2-21 is displayed.

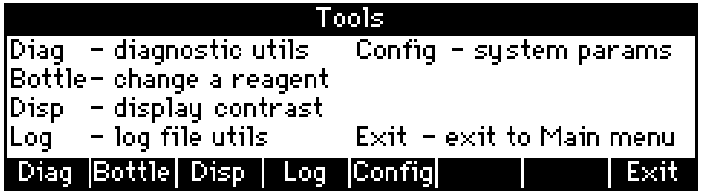


Figure 2-21 Tools Menu

2. Select **Bottle**.

   The Bottle Change Tool is displayed (see Figure 2-22).

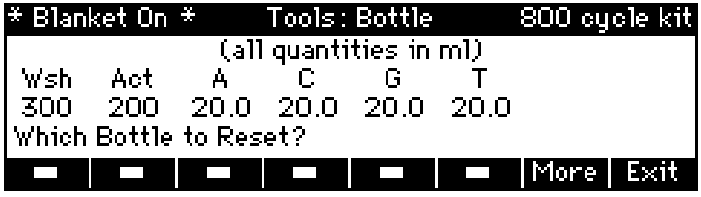


Figure 2-22 B-train Reagent Consumption Reset

3. Select **More**.

   The screen shown in Figure 2-23 is displayed.

Figure 2-23 A-train Reagent Consumption Reset

NOTE: *The blanket pressure is turned off when this screen is displayed. Do not display this screen any longer than is necessary.*

4. Remove the bottle seal and cap from the **WSH A** bottle. Transfer the contents to the 2000 mL plastic-coated bottle provided. Place it in the number 7 position in the reagent tray. Screw on the cap (line is labeled WA7).
5. Press the **WSH A** key on the instrument display to **Reset** the level of the Wsh A reservoir.

Install Deblock, Oxidizer, and Capping Reagents

To install the Deblock, Oxidizer, and Capping reagents:

1. Remove the bottle seal and cap from the **DBLK** bottle.
   - ***Model 8905*** —Screw the **DBLK** bottle into the **number 3** position in the front row of the lower reagent rack.
   - ***Model 8909*** —Transfer the contents of the DBLK bottle to a 1000 ml plastic-coated bottle provided. Place it in the **number 8** position in the reagent tray. Screw on the cap (line is labeled DB8).
2. Remove the bottle seal and cap from the **OX** bottle and screw it into the **number 2** position in the front row of the lower reagent rack in the reagent compartment (see Figure 2-20).

3. Remove the bottle seal and cap from the **CAP A** bottle and screw it into the **number 5** position in the lower row of the reagent rack.

4. Remove the bottle seal and cap from the **CAP B** bottle and screw it into the **number 6** position in the lower row of the reagent rack.

5. If the number 3 position is not to be used for a reagent, screw an empty bottle into the instrument cap.

   Make sure the bottles are firmly attached to the instrument cap. The bottles should be “finger tight.” Do not overtighten the bottles because this deforms the sealing O-rings and may cause gas pressure leakage.

6. Press the appropriate keys on the instrument display (see Figure 2-23) to **Reset** the level of the Deblock, Oxidizer and Capping reagent reservoirs.

Install Activator and Wash Reagent

To install the Activator and Wash reagents:

1. Press **More** until the screen shown in Figure 2-22 is displayed.

NOTE: *The blanket pressure is turned on and remains on while you are installing the amidites, wash and activator.*

---

2. Remove the bottle seal and cap from the **ACT** bottle and screw it into the **number 4** position in the lower row of the reagent rack (see Figure 2-20).

3. Remove the bottle seal and cap from the **WSH** bottle and screw it into the **number 1** position in the front row of the lower reagent rack.

   Make sure the bottles are firmly attached to the instrument cap. The bottles should be “finger tight.” Do not overtighten the bottles because this deforms the sealing O-rings and may cause gas pressure leakage.

4. Press the appropriate keys on the instrument display shown in Figure 2-22 to **Reset** the level of the Activator and Wash reagent reservoirs.

Install the Amidites

To install the amidites:

1. Dissolve the amidites as described below.

   Use the disposable syringes, needles, and the amidite diluent provided with the reagent kit, to reconstitute each amidite.

   - Fill a syringe with argon or nitrogen.
   - Pierce the septum on the amidite diluent bottle and push the gas into the bottle.
   - Withdraw enough diluent, as specified in Table 2-2, to make a 50 mg/mL solution.
   - Remove the needle from the syringe.
   - Expel the amidite diluent down the inside wall of the amidite bottle. Take care not to atomize the diluent.
   - Replace the cap on the amidite bottle.

Table 2-2 Amidite Diluent Volumes for 50 mg/mL Solution

Model	Number of Cycles at 0.2 μmole Scale	Amidite (g)	Diluent (ml)
8905	150	0.25	5.0
8909	400	0.5	10.0
	800	1.0	20.0

   - Agitate the solution thoroughly until all the amidite is completely dissolved.
   - Remove the cap from the amidite bottle.

2

2. Screw the amidite bottles into their respective (**A, C, G, T/U**) positions in the front row of the reagent rack (see Figure 2-20).

   Make sure the bottles are firmly attached to the instrument cap. The bottles should be “finger tight.” Do not overtighten the bottles because this deforms the sealing O-rings and may cause gas pressure leakage.

3. Press the appropriate keys on the instrument display shown in Figure 2-22 to **Reset** the levels of the Amidite reservoirs.

4. If you are installing amidites in the 5 though 9 reservoirs, press **More** until the screen shown in Figure 2-24 is displayed.

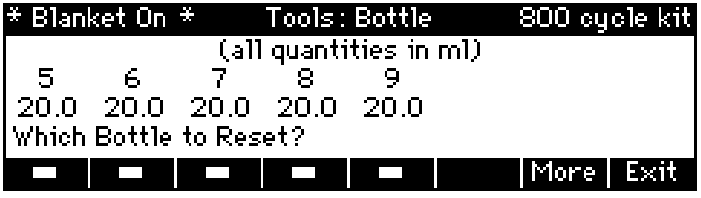


Figure 2-24 Model 8909 Amidite Consumption Reset

5. Screw the amidites into their respective (**5, 6, 7, 8, 9**) positions in the second row of the reagent rack (see Figure 2-20).

   Make sure the bottles are firmly attached to the instrument cap. The bottles should be “finger tight.” Do not overtighten the bottles because this deforms the sealing O-rings and may cause gas pressure leakage.

6. Reset the level of the reagent reservoirs that have been filled.

7. Install empty amidite bottles in any positions that are not being used.

NOTE: *All bottles are pressurized together. You must attach an empty bottle to any unused instrument position.*

2.4.3 Priming the System

When to prime

Prime the fluidics system **three times** for each column position after installing new reagents or after the instrument has been idle for more than 12 hours.

Priming

NOTE: *During the priming procedure, watch the waste line to ensure that every injector is delivering liquid.*

1. Install a union in place of the reaction columns (see Figure 2-25).

CAUTION

Do not install the column before you prime the reagent passages. The priming procedure pumps all the reagents through the column to waste, and would damage the column.

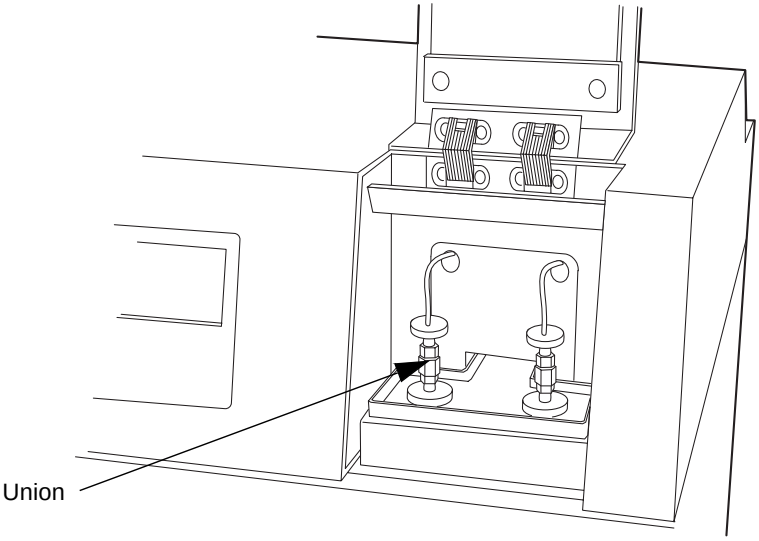


Figure 2-25 Install a Union in Place of the Reaction Column

2. In the Main menu, shown in Figure 2-11, select **Prime.**

   The Prime menu, shown in Figure 2-26, is displayed.

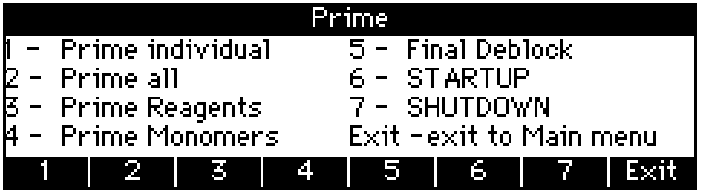


Figure 2-26 Prime Menu

3. Select **2 - Prime all**.

NOTE: *The use of the various options in the Prime menu is described in Section 3.5, Prime Menu.*

---

4. Select Column **1.**

   A message is displayed instructing you to replace the column with a union.

5. Press **OK** to continue after you install the union.

   All the reagent passages are primed sequentially (see Figure 2-27). During the automatic prime procedure, the current action is displayed on the screen and you may use the Stop key to abort the priming procedure.

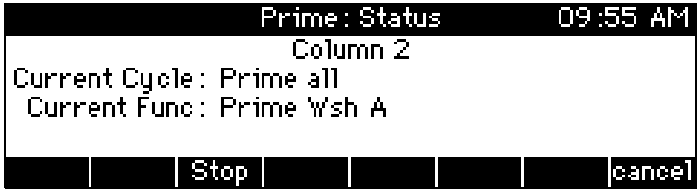


Figure 2-27 LCD Display During Priming

6. When the priming routine is complete, press **Cycle** to prime a second time.
7. When the priming routine is complete, press **Cycle** to prime a third time.
8. Press **cancel** to return to the Prime menu.

   The Prime menu, shown in Figure 2-26, is displayed.

9. If you are running a synthesis on both columns, select **2 - Prime all**.
10. Select Column **2 and prime three times.**
11. Select **Exit** to return to the Main menu.

2.4.4 Running the Pneumatic Diagnostics

Periodically, before starting a synthesis, you should run the **Diagnostics** routines to check the pneumatic system for leaks.

To run the Leak Test:

1. Select **Tools** in the Main menu.

   The Tools menu (see Figure 2-21) is displayed.

2. Select **Diag** (Diagnostic utilities).

   The diagnostics menu shown in Figure 2-28 is displayed.

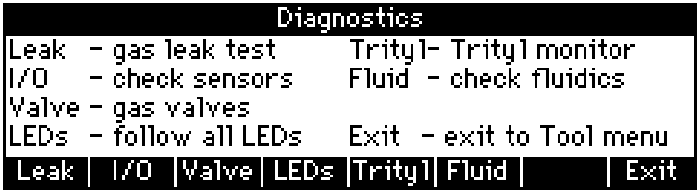


Figure 2-28 Diagnostics Menu

3. Select **Leak**.

4. Select **Start.**

   The system performs an automatic test of the High and Low pressure systems (see Section 3.6.1, Diagnostic Routines, for more information). On completion of the test, the result is shown on the screen (see Figure 2-29).

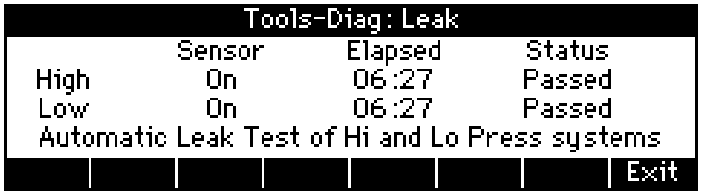


Figure 2-29 Screen at End of Successful Leak Test

The test is completed in 3 minutes but it continues to run until you exit the screen.

5. Press **Exit** to return to the Main menu.

2.5 Starting the Synthesis

Once the sequence has been entered and the reagents have been loaded, the next steps are to:

- Specify the synthesis parameters
- Install the column
- Start the synthesis

2.5.1 Specifying the Synthesis Parameters

To specify the synthesis parameters:

1. Select **Seq** from the Main menu.
2. Select **Run** from the Sequence menu (see Figure 2-15).
3. Select the sequence to be used (see Figure 2-16).

   The screen shown in Figure 2-30 is displayed.

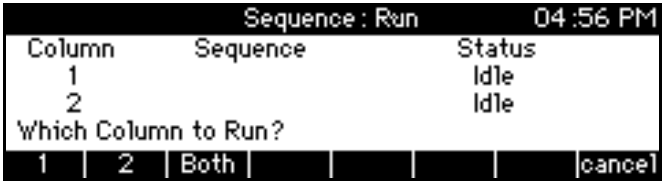


Figure 2-30 Column Selection

4. Select the column or columns on which to run the sequence.

NOTE: *You may start the same synthesis on both columns. However, if you wish to run different syntheses on column one and column two, you must repeat this procedure to select and start the second synthesis.*

If you select column 1, the screen shown in Figure 2-31 is displayed.

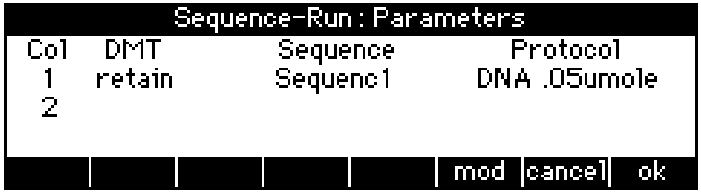


Figure 2-31 Synthesis Parameters Screen

5. Make sure that the synthesis parameters are correct for the synthesis. The available parameters are described in Table 2-3.

Table 2-3 Synthesis Parameters

Parameter	Description
Col	The column (1 or 2) to be used.
DMT	Remove or retain the 5' DMT protecting group. ***NOTE:*** You may wish to retain the 5' DMT to observe the final trityl color. In this event, use the Final Deblock option in the Prime menu to manually remove the 5' DMT at the end of the synthesis (see Section 3.5.5, Final Deblock).
Sequence	The name of the sequence to be run.
Protocol	The protocol to be used. The options for DNA are: 0.05 µmole 0.2 µmole 1.0 µmole 15.0 µmole

6. If the running parameters are incorrect, select **mod.**

   The screen shown in Figure 2-32 is displayed.

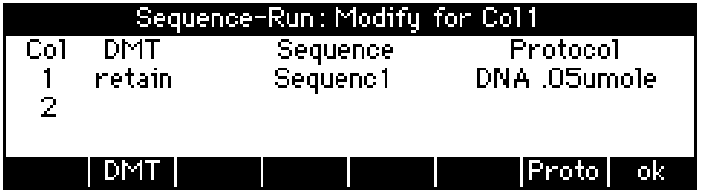


Figure 2-32 Modify Parameters

7. At this time, you may:

   - Press **DMT** to toggle between retaining or removing the 5' DMT protecting group.
   - Press **Proto** to cycle through the list of protocols available for the selected chemistry.

NOTE: *You may change the default chemistry for the current profile by modifying the User Profile (see Section 3.6.6, Specifying a User Profile).*

---

8. Press **ok** to accept the running parameters.
9. You are prompted to install the column.

CAUTION

Changing reagent bottles during a synthesis is not recommended. Check the reagent resources prior to initiating a synthesis to make sure that there is a sufficient supply.

2.5.2 Checking Reagent Resources

Check your reagent resources before installing the column and starting the synthesis.

1. Press **ok** until the Main menu is displayed.
2. Press **Stat**. The status screen shown in Figure 2-33 is displayed.

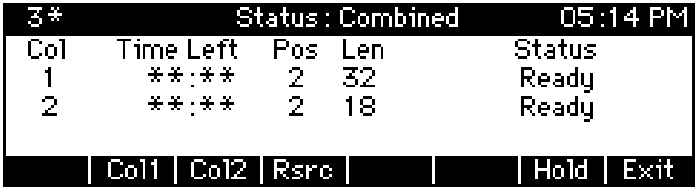


Figure 2-33 Combined Column Status

3. Press **Rsrc**. The screen shown in Figure 2-34 is displayed. The volumes of reagents required (req) for the synthesis and the volumes currently in the reservoirs (cur) are displayed.

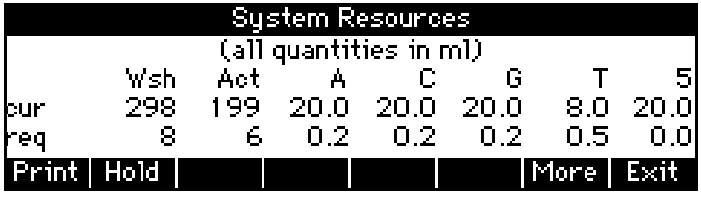


Figure 2-34 Reagent Resources

- If you have sufficient reagents in the reservoirs, install the column (see Section 2.5.3, Installing the Column) and proceed with the synthesis.
- If you do not have sufficient reagents, replenish the required bottles (see Section 2.4.2, Installing the Reagents) and prime the reagent passages (see Section 2.4.3, Priming the System) before you install the column.

2.5.3 Installing the Column

CAUTION

Do not install the reaction column before you run the prime routine. The column may be damaged irreversibly.

When you specify the synthesis parameters, you are prompted to install the appropriate column (see Figure 2-35).

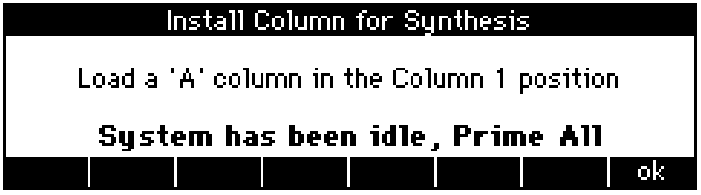


Figure 2-35 Install a Column Prompt

NOTE: *If the system has been idle for 12 hours or more or has been shut off, you are prompted to prime the system. Install a union in place of the column and prime each column position* ***three times****.*

The columns are bidirectional and can be attached in any direction.

If you are using a MemSyn device, place it on the instrument with the printed side of the label facing up.

To install the reaction column (see Figure 2-36):

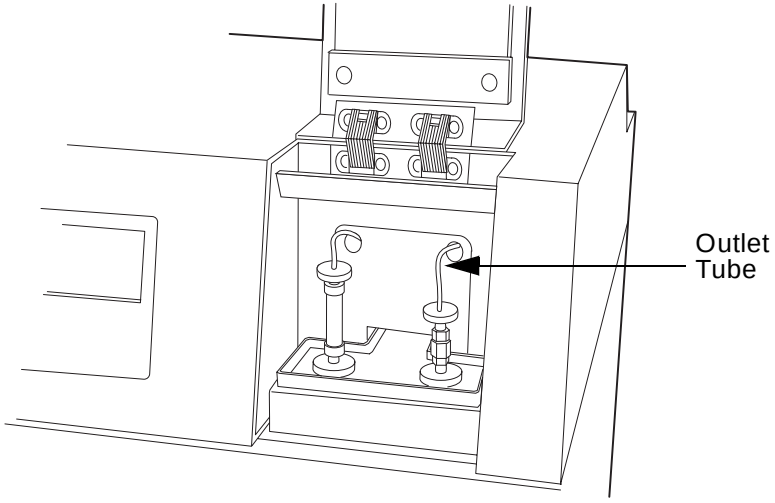


Figure 2-36 Installing the Column

1. Firmly insert the column onto the male Luer fitting in the reaction chamber. Use a slight twisting motion—about one quarter turn.
2. Insert the upper column fitting into the top of the column. Use a slight twisting motion—about one quarter turn.

NOTE: *If you are running a synthesis on only one column, install a union in place of the second column to prevent leakage from inlet and outlet tubes.*

2.5.4 Starting the Synthesis

To start the synthesis:

1. Select **Exit** until the Main menu, shown in Figure 2-37, is displayed. The Start option is now available.

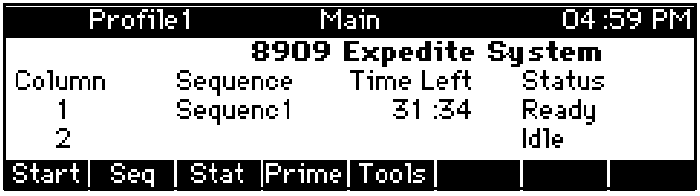


Figure 2-37 Main Menu—Ready to Begin a Synthesis

2. Press **Start** to begin the synthesis on Column 1.

NOTE: *If a synthesis is already running, the screen shown in Figure 2-38 is displayed. In this event, press* **ok** *to start the synthesis.*

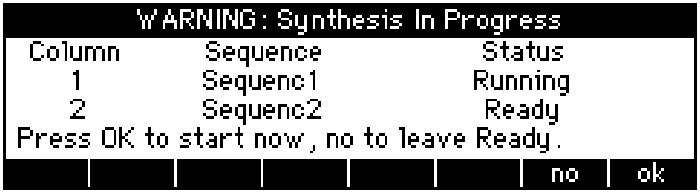


Figure 2-38 Starting a Second Synthesis

2.6 Running the Synthesis

During a synthesis run, the software monitors the progress (see Figure 2-39) and displays the time left until the synthesis is completed.

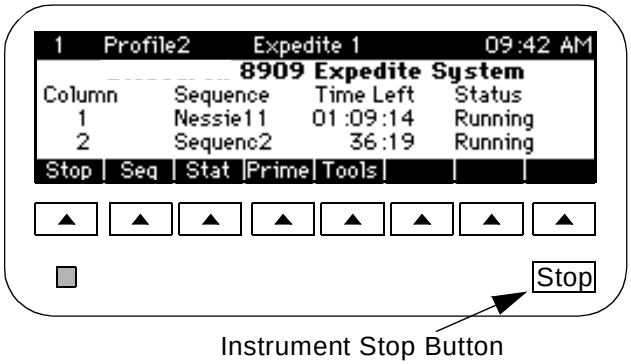


Figure 2-39 Screen During a Run

Stop Key

You may press the Instrument Stop key, shown in Figure 2-39, at any time. When you press this key, all pumping operations are halted immediately and remain halted until you press the **Start** key in the Main menu.

NOTE: *Pausing a synthesis for extended periods in the middle of a cycle may be detrimental to the synthesis. If you use the Stop key, restart the synthesis as soon as possible. If you wish to pause a synthesis to replenish reagents, use the Hold option (see* *"Interrupting the Synthesis" on page 2-50**).*

Monitoring the Synthesis

During the synthesis, the Main menu displays the status of both columns and the estimated time (hours:minutes:seconds), until completion of the synthesis.

The Stat option on the Main menu displays more detailed information on the synthesis. When you select **Stat,** the screen shown in Figure 2-40 is displayed.

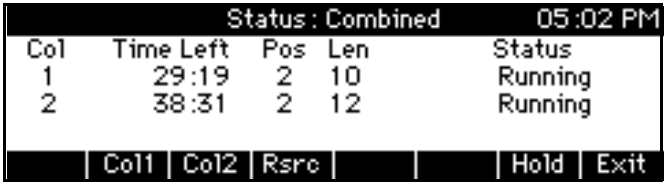


Figure 2-40 Synthesis Status Screen

The Col1 and Col2 options provide more detailed information (see Section 3.4.1, Displaying System Information).

Interrupting the Synthesis

On occasion, you may want to interrupt the synthesis. In this event, select **Hold** on the Status: Combined menu (Figure 2-40). The instrument halts the synthesis on each column at the end of the current cycle (usually within 5 minutes). This is the safest point to interrupt the synthesis. The column is washed with acetonitrile before the instrument halts. The synthesis remains halted until you press **No Hold** to restart the synthesis (see Section 3.4.2, Interrupting a Synthesis).

2.6.1 Replenishing Bottles During a Synthesis

CAUTION

Changing reagent bottles during a synthesis is not recommended. Check the reagent resources prior to initiating a synthesis to make sure that there is a sufficient supply.

If a reagent must be changed during a synthesis, use the following procedure to install one reagent at a time.

1. Select **Hold** on the Status: Combined menu (Figure 2-40) to stop the instrument. The instrument halts the synthesis on each column at the end of the current cycle (usually within 5 minutes). This is the safest point to interrupt the synthesis.

   CAUTION

   Make sure the synthesis has halted on both columns before proceeding.

2. Remove the column or columns and replace them with a union. Remember to attach the outlet tube.

3. In the Main menu, select **Tools.**

4. In the Tools menu, select **Bottle**. The Bottle Change Tool, shown in Figure 2-41 is displayed.

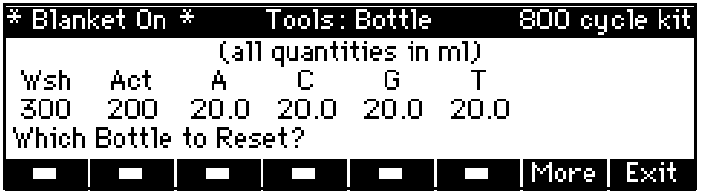


Figure 2-41 Bottle Change Tool

5. Collect the required reagents and place them in the vicinity of the instrument.
6. Remove the empty reagent bottle to be changed from the instrument.
7. Remove the bottle seal and cap from the new reagent bottle and screw the bottle into the appropriate position in the instrument.

   Make sure the bottles are firmly attached to the instrument cap. The bottles should be “finger tight.” Do not overtighten the bottles because this deforms the sealing O-rings and may cause gas pressure leakage.
8. On the instrument display (see Figure 2-41), locate the reagent that has just been replenished and reset its level.
9. Repeat steps 6 through 8 for the remaining reagents. Remember to reset the level of each reagent bottle.

Prime the Reagent Passages

To prime the individual reagent passages:

1. From the Main menu select **Prime**.
2. Select **Prime individual** from the Prime menu.

***NOTE**: To prime multiple reagent passages, you can select Prime all.*

3. Select **Column 1.**

   The screen shown in Figure 2-42 is displayed.

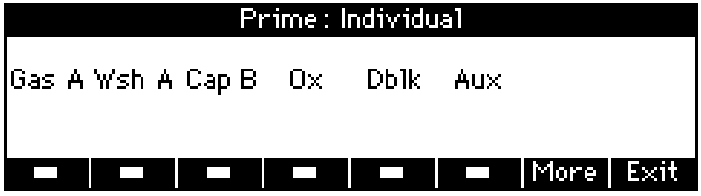


Figure 2-42 Prime Individual Menu

4. Select the reservoir to prime.

   If the reagent is not displayed, press more to display additional reagents.

5. Press **Cycle** until you have primed three times.

6. Press **Cancel** and select another reagent if necessary.

7. Repeat step 4 through step 6 to prime all reagents that you change.

8. Select **Wsh A** to rinse the A reagent train.

   ***NOTE**: 8905 systems have only Wsh.*

9. Press **Cancel**, then press **More**. Press **Wsh** to rinse the B reagent train.

10. Press **Cancel**, then press **Exit** until the Prime menu is displayed.

11. Repeat step 4 through step 10 for Column 2.

12. Press **Exit** to return to the main menu.

13. Remove the union and replace the column or columns in the position from which they were removed and attach the outlet tube.

14. Press **Start** to resume the synthesis.

2.6.2 Post-synthesis Column Removal

When the synthesis is complete, use the following procedure to remove the column from the instrument:

1. Remove the outlet tube from the top of the column by pulling the fitting with a slight twist.
2. Remove the column from the bottom fitting by pulling it with a slight twist.
3. Insert a union in place of the column.

NOTE: *Whenever the column is removed from the instrument, install a union to ensure that any leakage or priming is directed to the waste.*

2.7 Post-synthesis Procedures

Once the synthesis is complete, the oligomer must be cleaved from the support and fully deprotected.

You may use either the Syringe Column or the Sealed Tube method to isolate the product from the support and deprotect it. The time required to perform cleavage and deprotection depends on the monomers used in the synthesis. Cleavage and deprotection procedures for Expedite monomers are faster and use milder conditions than the procedures for standard monomers.

WARNING

Wear a lab coat, gloves and eye protection when performing the procedures described in this section. Adequate ventilation is essential and working under a fume hood is recommended.

AVERTISSEMENT

Portez une blouse, des gants et des lunettes de protection lorsque vous suivez les protocoles décrits dans cette section. Une ventilation adéquate est indispensable et il est recommandé de travailler sous une hotte.

Cleavage and Deprotection Reagent

Concentrated ammonium hydroxide (30% aqueous NH_4OH) is required for cleavage and deprotection.

NOTE: *Use fresh ammonium hydroxide (open less than 2 months). To ensure a concentrated solution after opening, aliquot the ammonium hydroxide in small volumes, cap tightly, and store in a freezer.*

2.7.1 Standard Phosphoramidite Monomers

Syringe Column Cleavage and Deprotection

To perform syringe column cleavage and deprotection of oligomers synthesized with standard monomers:

1. Remove the column or MemSyn device from the instrument (see Section 2.6.2, Post-synthesis Column Removal).
2. With the plunger fully depressed into the barrel, attach a slip-tip Luer syringe to one end of the column or MemSyn device (see Figure 2-43).
   - Use a 1, 3, or 5 mL syringe for 0.05, 0.2 or 1.0 μmol scales.
   - Use 5 mL syringes for 15 μmol scale.

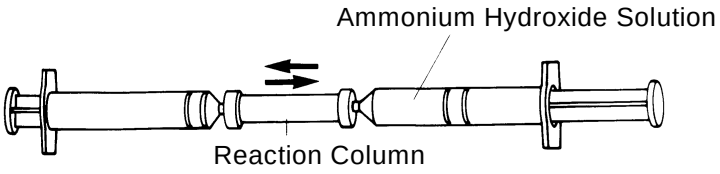


Figure 2-43 Syringe Column Cleavage and Deprotection

3. Draw 1 mL of fresh NH_4OH (5 mL for 15 μmol scale) into a second Luer tip syringe.
4. Attach the syringe to the other end of the column or MemSyn device.
5. Depress the syringe plunger to force the solution back and forth over the support in the column or MemSyn device. Repeat three or four times to ensure that the support in the column or MemSyn device is fully saturated.

6. Let the column or MemSyn device/syringe assembly rest at room temperature for 45 minutes.
7. Push the solution back and forth three or four times.
8. Let the assembly rest for another 45 minutes.
9. Draw all the solution into one of the syringes.

   This solution contains:

   - Product oligomer
   - Shorter failure sequences
   - By-products of phosphorus deprotection

10. Carefully remove the syringe from the column or MemSyn device (ammonium hydroxide is very volatile).
11. Deposit the solution into a screw-cap tube or vial.
12. Securely cap the tube.
13. Perform one of the following:

    - Heat the tube at 55 ^{O}C for 6 to 8 hours or overnight. (Overnight is recommended for sequences with > 25% G monomers.)
    - Let the reaction proceed at room temperature for 24 to 48 hours. (Forty-eight hours is recommended for sequences with > 25% G monomers.)

WARNING

Cool the tube before opening. Heated NH_4OH is caustic and will spray if you open the tube before it is cool.

AVERTISSEMENT

Laissez refroidir le tube avant de l'ouvrir. L'ammo-niaque chauffée est caustique et risque de jaillir si vous ouvrez le tube avant qu'il n'ait refroidi.

14. Cool the tube to room temperature.
15. If necessary, decant the DNA containing supernatant into a tube suitable for evaporation.

Sealed Tube Cleavage and Deprotection

Perform the following steps when using the sealed tube cleavage and deprotection method.

1. Remove the column or MemSyn device from the instrument (see Section 2.6.2, Post-synthesis Column Removal).
2. If you are using a column:

   Pry the end crimp off one end of the column and remove the cap and filter.

   Pour the CPG support from the column into a small screw cap container.

   - A small screw cap vial is suitable for 0.05, 0.2 or 1.0 μmole syntheses.
   - A 15 mm x 125 mm screw cap vial is suitable for 15 μmole syntheses.

   If you are using a MemSyn device, remove the MemSyn and place it in a small screw cap container as described above.

3. Add NH_4OH.
    - For 0.05, 0.2 or 1.0 μmole syntheses, add 1 to 2mL.
    - For 15 μmole syntheses, add 6 mL.
4. Agitate the tube to suspend the CPG or MemSyn.
5. Let the tube stand for 90 minutes at room temperature.
6. If you are using CPG support, separate the NH_4OH solution containing the cleaved CPG from the support:
    - Centrifuge the tube.
    - Decant the NH_4OH solution containing the cleaved DNA product and place it in another tube.
    - Add 0.5 mL NH_4OH to wash the solid support in the original tube.
    - Mix the support and NH_4OH and centrifuge.
    - Decant the NH_4OH solution containing the DNA product into the tube containing the first decant.
    - Securely cap the tube.

    If you are using a MemSyn device, you can remove the membrane from or leave the membrane in the container. The membrane has no affect on the deprotection.
7. Perform one of the following:
    - Heat the tube at 55°C for 6 to 8 hours or overnight. (Overnight is recommended for sequences with > 25% G monomers.)
    - Let the reaction proceed at room temperature for 24 to 48 hours. (Forty-eight hours is recommended for sequences with > 25% G monomers.)

WARNING

Cool the tube before opening. Heated NH_4OH is caustic and will spray if you open the tube before it is cool.

AVERTISSEMENT

Laissez refroidir le tube avant de l'ouvrir. L'ammo-niaque chauffée est caustique et risque de jaillir si vous ouvrez le tube avant qu'il n'ait refroidi.

8. Cool the tube to room temperature.
9. If necessary, decant the DNA containing supernatant into a tube suitable for evaporation.

2.7.2 Expedite Phosphoramidite Monomers

The sealed tube method of cleavage and deprotection for oligomers synthesized with the Expedite phosphoramidite monomers requires a minimum of 15 minutes. The syringe column method requires 2 hours.

Sealed Tube Cleavage and Deprotection

Perform the following steps when using the tube cleavage and deprotection method:

***NOTE**: You can also perform sealed tube cleavage and deprotection using MemSyn Nucleic Acid Synthesis Devices. Refer to the MemSyn Operating Instructions for information on removing and opening the device.*

1. Remove the column from the instrument (see Section 2.6.2, Post-synthesis Column Removal).
2. Pry the end crimp off one end of the column and remove the cap and filter.

3. Pour the CPG support into a small screw cap container.
    - A small screw cap vial is suitable for 0.05, 0.2 or 1.0 μmole syntheses.
    - A 15 mm x 125 mm screw cap vial is suitable for 15 μmole syntheses.
4. Add NH_4OH.
    - For 0.05, 0.2 or 1.0 μmole syntheses, add 1 to 2mL.
    - For 15 μmole syntheses, add 5 mL.
5. Agitate the tube to suspend the CPG.
6. Perform one of the following:
    - Heat the tube at 55 ^{O}C for 15 minutes.
    - Let the reaction proceed at room temperature for 2 hours.

WARNING

Cool the tube before opening. Heated NH_4OH is caustic and will spray if you open the tube before it is cool.

AVERTISSEMENT

Laissez refroidir le tube avant de l'ouvrir. L'ammo-niaque chauffée est caustique et risque de jaillir si vous ouvrez le tube avant qu'il n'ait refroidi.

---

7. Cool the tube to room temperature.
8. Centrifuge the tube and decant the DNA containing supernatant into a tube suitable for evaporation.

9. Add 0.5 mL NH_4OH to wash the solid support in the original tube.
10. Mix the support with NH_4OH and centrifuge.
11. Decant the NH_4OH solution containing the DNA product into the tube containing the first decant.

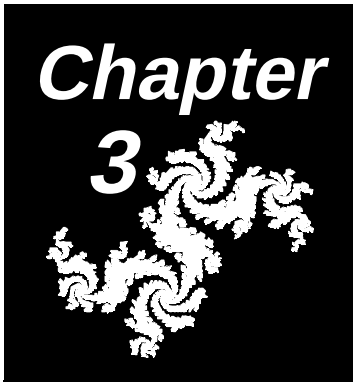
Syringe Column Cleavage and Deprotection

To perform syringe column cleavage and deprotection of oligomers synthesized with Expedite monomers:

1. Remove the column from the instrument (see Section 2.6.2, Post-synthesis Column Removal).
2. With the plunger fully depressed into the barrel, attach a slip-tip Luer syringe to one end of the column (Figure 2-43).
    - Use a 1, 3, or 5 mL syringe for 0.05, 0.2 or 1.0 μmol scales.
    - Use a 5 mL syringe for 15 μmol scale.
3. Draw 1 mL of fresh NH_4OH (5 mL for 15 μmol scale) into a second Luer tip syringe.
4. Attach the syringe to the other end of the column.
5. Depress the syringe plunger to force the solution back and forth over the support in the column. Repeat three or four times to ensure that the support in the column is fully saturated.
6. Let the column/syringe assembly rest at room temperature for 60 minutes.
7. Push the solution back and forth three or four times.
8. Let the assembly rest for 60 minutes.
9. Draw all the solution into one of the syringes.
10. Remove the syringe from the column.
11. Deposit the solution into a screw-cap tube or vial suitable for evaporation.

Purification and Analysis

The required purity of the final product, and therefore the procedures used to separate out the desired product, depend on the intended use for the oligomer.



3 Software Reference

This chapter contains the following sections:

3.1 Software Overview

The Expedite Nucleic Acid Synthesis System is controlled by menu-driven software which:

- Allows independent single or dual column operation.
- Provides full sequence editing facilities.
- Stores 63 sequences up to 250 bases in length in each of eight user profiles, for a total of 504 sequences.
- Calculates and monitors reagent resources for the synthesis.
- Monitors the synthesis and displays the current status and estimated time of completion.
- Provides extended diagnostics for troubleshooting.
- Provides tools for priming the instrument and various manual functions.
- Monitors for error conditions.
- Generates a log of all activities.
- Notifies you, after an idle period, of recommended procedures that should be performed before a new synthesis is started.

A flow diagram of the hierarchical menu structure is shown in Figure 3-1. Different functions are assigned to the soft keys displayed at the bottom of each screen. To select a menu option, press the soft key directly below the desired option.

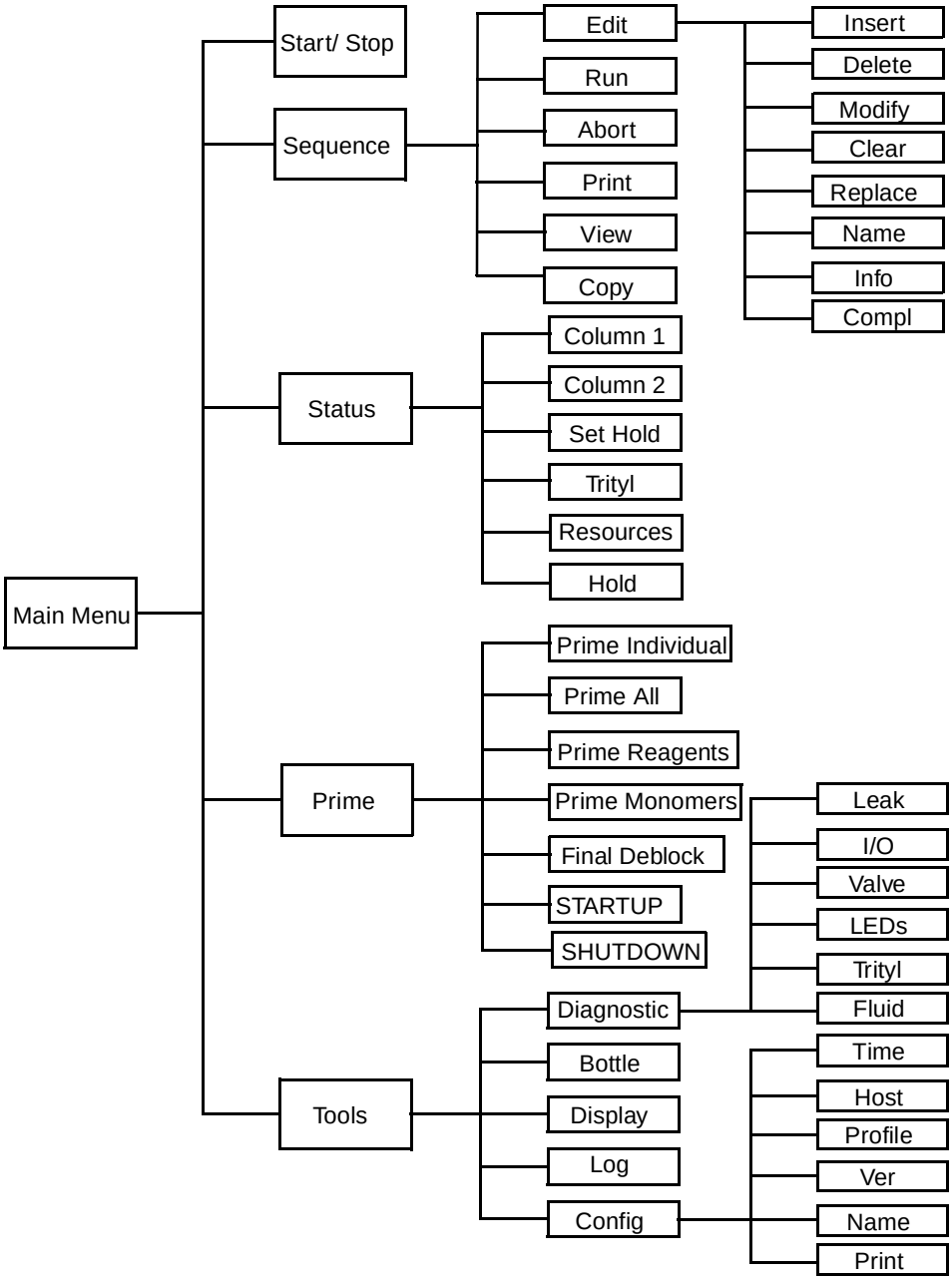


Figure 3-1 Overview of the Menus

3.2 Main Menu

When the system is initialized (see Section 2.2, Powering Up the System), the Main menu is displayed. The Main menu, shown in Figure 3-2, provides access to all the utilities used to set up a synthesis and operate the instrument. The Main menu displays the following:

- The current sequence name
- The column status (Idle, Running, Done, Halted)
- The instrument address (used to distinguish multiple instruments connected to a remote host)
- Profile name
- Instrument name
- The current time
- The time left to complete the synthesis (synthesis countdown times)
- The functions defined for the soft keys

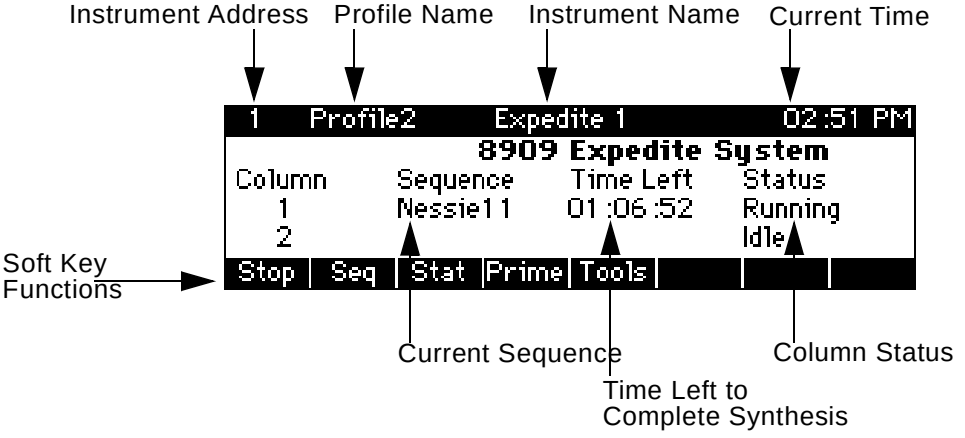


Figure 3-2 Main Menu

NOTE: *An asterisk (*) beside the Instrument Address indicates that the instrument is communicating with the host workstation.*

Main Menu Keys

The functions that are assigned to the soft keys in the Main menu are described in Table 3-1.

Table 3-1 Main Menu Keys

Key #	Label	Function
1	Start	Starts the instrument. When you select Start, all active syntheses will proceed. ***NOTE:*** *This key is not displayed until a sequence is selected to be run.*
	Stop	Halts the instrument immediately. ***NOTE:*** *This key is available as soon as the synthesis is started.*
2	Seq	Create, edit, and run sequences. See Section 3.3, Sequence Menu for details.
3	Stat	Monitors the current syntheses. See Section 3.4, Status Menu, for details.
4	Prime	Manually prime the fluidics system. Perform final deblock (manually remove 5' DMT). Wash out the instrument. See Section 3.5, Prime Menu, for details.
5	Tools	Provides access to the maintenance log and the various tools that facilitate operation and troubleshooting of the instrument. See Section 3.6, Tools Menu, for details.

3.3 Sequence Menu

Use the Sequence menu to:

- Create or edit a sequence
- Run a synthesis
- Abort a synthesis
- Print a sequence
- View stored sequences
- Copy an existing sequence for further editing

When you select Seq from the Main menu, the screen shown in Figure 3-3 is displayed.

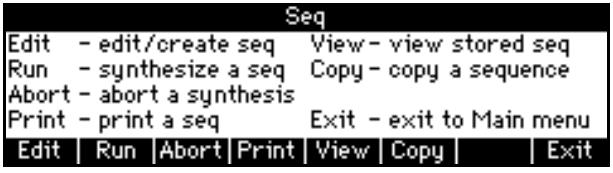


Figure 3-3 Sequence Menu

Sequence Menu Keys

The functions that are assigned to the soft keys in the Sequence menu are described in Table 3-2.

Table 3-2 Sequence Menu Keys

Key #	Label	Function
1	Edit	Provides access to the Sequence Editor which is used to create or edit a sequence. See Section 3.3.1, Using the Sequence Editor.
2	Run	To start the synthesis of a sequence that has been created with the sequence editor. See Section 3.3.2, Running a Sequence.
3	Abort	To abort a synthesis after the instrument has been halted. See Section 3.3.3, Aborting a Synthesis.
4	Print	To print a sequence and its summary information. See Section 3.3.4, Printing a Sequence.
5	View	To view stored sequences. This is a read-only mode for viewing the currently stored synthesis data. See Section 3.3.5, Viewing a Sequence.
6	Copy	To copy an existing sequence to another slot for further editing. See Section 3.3.6, Copying a Sequence.
8	Exit	To return to the Main menu.

Sequence Selection

The Sequence-Select menu, shown in Figure 3-4, is displayed when you select any option on the Sequence menu (except Abort or Exit).

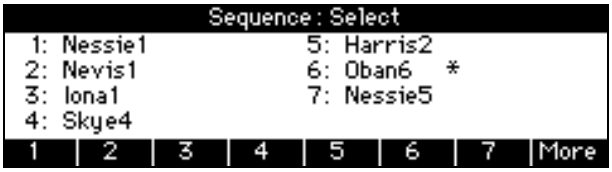


Figure 3-4 Sequence Select Menu

Press the appropriately numbered key at the bottom of the screen to select the desired sequence. Selecting More allows you to view more sequences if present.

NOTE: *The asterisk (*) beside Sequence 6: Oban6 indicates that this was the last sequence to have been edited.*

3

Sequence Names

As many as sixty-three sequences may be stored with an instrument generated or a user supplied name. In the sequence selection screen, instrument generated names are displayed in upper and lower case and user generated names are displayed in upper and lower case as specified by the user (see "Naming a Sequence" on page 3-23).

3.3.1 Using the Sequence Editor

Use the Sequence Editor, shown in Figure 3-5, to create and modify sequences.

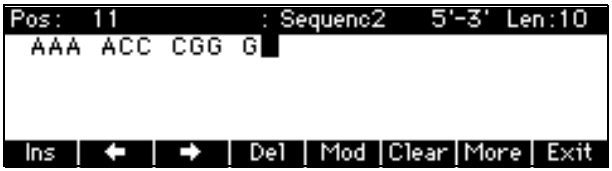


Figure 3-5 Sequence Editor—First Screen

When you are editing a sequence, the following information is displayed on the top line of the screen:

- Cursor location (Pos: #)
- Mode of entry (Ins/Repl/Mix) (see Figure 3-6)
- Sequence name
- Direction of sequence entry (5'-3' or 3'-5')
- The length of the sequence (Len: #)

Sequences are displayed in the center of the screen with the character groupings specified in the User Profile facility (see Section 3.6.6, Specifying a User Profile).

Sequence Editor Keys

In the Sequence Editor, the first two screens contain the editing options that are described in Table 3-3. The remaining screens contain entry mode options that are described in Table 3-4.

Entering a Sequence

When you select Insert or Replace, the sequence entry mode screen is displayed (Figure 3-6).

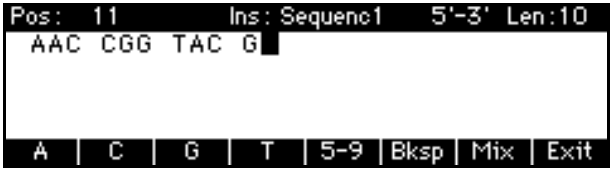


Figure 3-6 Sequence Editor—Entry Mode

The function keys are re-assigned (see Table 3-4) to allow you to enter bases into the displayed sequence.

The default entry mode is the 5' to 3' direction. To enter sequences in the 3' to 5' direction, modify the User Profile (see Section 3.6.6, Specifying a User Profile).

3

Upper case and lowercase base designators

Sequences are case-sensitive. Uppercase designators represent different residues than lowercase designators.

Chemistry	Uppercase	Lowercase
DNA	DNA	Thio*
Thio	Thio*	DNA
RNA	RNA	DNA

* Uses the auxiliary bottle for thioating.

Changing case

To change the case of a base designator, enter the sequence (uppercase by default), use the arrow keys to select the designators to change, then press **Mod**.

Table 3-3 Sequence Editor Keys

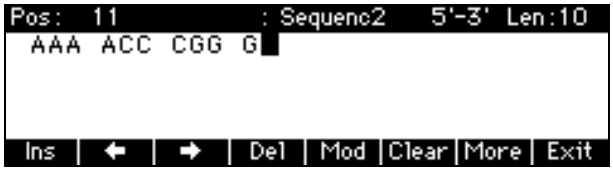
Key #	Label	Function
First Screen Keys		 ***Figure 3-7 Sequence Editor—First Screen***
1	Ins	Selects the Insert mode of sequence entry. When you select Insert, the screen shown in Figure 3-6 is displayed and you can enter bases into the sequence.
2	←	Moves the cursor one place to the left.
3	→	Moves the cursor one place to the right.
4	Del	Deletes the character at the cursor.
5	Mod	Depending on the selected Chemistry, enables you to specify a modified cycle. See "Modifying a Sequence" on page 3-19.
6	Clear	Deletes the entire sequence. When you select Clear a second screen is displayed and you must select one of the following: • **Yes**—delete the sequence • **Cancel**—do not delete the sequence This protects against accidental deletion of a sequence.
7	More	Displays the second sequence editor screen with additional menu choices.

Table 3-3 Sequence Editor Keys (Continued)

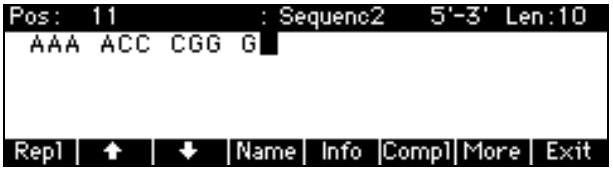
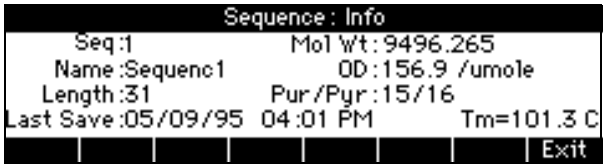
Key #	Label	Function
8	Exit	Returns you to the Sequence Editor menu. When you select this option to exit the Sequence Editor, a new screen is displayed and you are given the opportunity to select one of the following: • **Yes**—Save the sequence • **No**—Abandon changes made during the edit session • **Cancel**—Return to the Sequence Editor
Second Screen Keys		 ***Figure 3-8 Sequence Editor—Second Screen***
1	Replace	Selects the replace mode of sequence entry.
2	↑	Moves the cursor up one row, scrolling the display if appropriate.
3	↓	Moves the cursor down one row, scrolling the display if appropriate.
4	Name	Allows you to give the sequence a name. The procedure for naming a sequence is described in "Naming a Sequence" on page 3-23.

Table 3-3 Sequence Editor Keys (Continued)

Key #	Label	Function
5	Info	Displays the screen shown in Figure 3-9.  ***Figure 3-9 Sequence Information Screen*** This screen provides the following information about the displayed sequence: • Sequence number and name • Sequence length • Molecular weight and OD (see "Molecular Weight and OD Calculation" on page 3-24) • Purine to pyrimidine ratio • Melting temperature (see "Melting Point Calculation" on page 3-24) • Date and time of last save
6	Compl	Displays the complement of the displayed sequence.
7	More	Displays the first sequence editor screen.
8	Exit	Returns you to the Sequence Editor menu.

Entry Mode Keys

The functions that are assigned to the soft keys in Entry Mode are described in Table 3-4.

Table 3-4 Entry Mode Keys

Key #	Label	Function
1	A	Selects the reagent in the Adenosine monomer reservoir position.
2	C	Selects the reagent in the Cytidine monomer reservoir position.
3	G	Selects the reagent in the Guanosine monomer reservoir position.
4	T (DNA)	Selects the reagent in the Thymidine monomer reservoir position (DNA, THIO, and USER chemistry).
	U (RNA)	Selects the reagent in the Uridine monomer reservoir position (RNA chemistry only).
5	**5-9** 8909	Displays the second base selection screen.
	5 8905	Selects the reagent in the #5 monomer reservoir position.
6	Bksp	Deletes the base at the left of the cursor.
7	Mix	Provides entry to Mixed Site Mode. The procedure for entering mixed sites is described in "Modifying a Sequence" on page 3-19.
8	Exit	Returns you to the previous menu.
Second Screen Keys (Model 8909 only)		
1	5	Selects the reagent in the #5 monomer reservoir position.

Table 3-4 Entry Mode Keys (Continued)

Key #	Label	Function
2	6	Selects the reagent in the #6 monomer reservoir position.
3	7	Selects the reagent in the #7 monomer reservoir position.
4	8	Selects the reagent in the #8 monomer reservoir position.
5	9	Selects the reagent in the #9 monomer reservoir position.

Entry Modes

There are two modes of base entry, Insert and Replace (overstrike).

Insert Mode Ins	Insert mode is available when you enter the Sequence Editor. In Insert mode, all entered bases are inserted into the sequence to the left of the cursor. Insert mode is suitable for entering new sequences and adding bases to an existing sequence.
Replace Mode Repl	Replace mode is available when you select **More** to show additional Sequence Editor options. In Replace mode, all entered bases are placed into the sequence at the current location of the cursor over-striking any existing character. Replace mode is useful for editing an existing sequence.

Mixed Site Entry

Dependent on the mixture you select, equal volumes are drawn from the nucleotide reservoirs and delivered to the column. When you select Mix, the screen shown in Figure 3-10 is displayed.

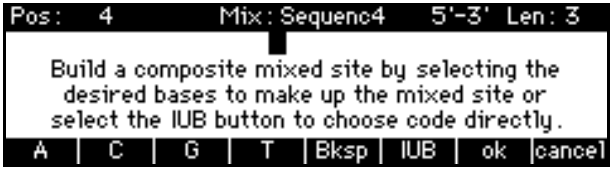


Figure 3-10 Sequence Editor—Mixed Base Entry Mode

The functions that are assigned to the soft keys to enable you to build a composite site are described in Table 3-5.

Table 3-5 Mixed Site Entry—Key Assignments

Key #	Label	Function
1	A	Inserts Adenosine in the mixed site.
2	C	Inserts Cytidine in the mixed site.
3	G	Inserts Guanosine in the mixed site.
4	T (DNA)	Inserts Thymidine in the mixed site if DNA, THIO, or USER chemistry is selected in the user profile.
	U (RNA)	Inserts Uridine in the mixed site if RNA chemistry is selected in the user profile.
5	Bksp	Deletes the character immediately preceding the cursor.
6	IUB	Causes the display of additional soft key functions that enables you to enter the appropriate IUB code directly. The IUB codes are listed in Table 3-6.
7	OK	Returns you to the previous screen and inserts the mixed site in the sequence.
8	Cancel	Returns you to the previous screen without inserting the mixed site in the sequence.

NOTE: *If you select mixed sites, the system uses equal percentages of each monomer. For example, if you select AC, the system uses 50% A and 50% G. If you select ACG, the system uses 33% A, 33% C, and 33% G. You cannot enter lowercase letters for mixed sites.*

IUB Codes

The IUB (International Union of Biochemistry) codes for bases and mixed bases are listed in Table 3-6.

Table 3-6 IUB Group Codes for Incompletely Specified Bases

Code	Bases Coded	Complement	Comment
A	A	T	Adenine
C	C	G	Cytosine
G	G	C	Guanine
T	T	A	Thymine (DNA only)
U	U	A	Uracil (RNA only)
R	A, G	Y	puRines
Y	C, T/U	R	pYrimidines
M	A, C	K	aMino
K	G, T/U	M	Keto
S	G, C	S	Strong (3H-bonds)
W	A, T/U	W	Weak (2H-bonds)
H	A, T/U, C	D	not G
B	G, T/U, C	V	not A
V	G, A, C	B	not T/U
D	G, A, T/U	H	not C
N	G, A, T/U, C	N	aNy

***NOTE**: You cannot enter lowercase letters when using IUB codes.*

Modifying a Sequence

Use the **mod** key to change the designation of the selected base to perform more complex synthetic tasks.

Modified cycles in the standard protocols allow you to:

- Use extended coupling times for monomer 5 by entering an X in the sequence
- Perform base-specific thioation of DNA (8909 only) by entering lowercase base designators in a DNA protocol
- Synthesize DNA/RNA hybrids (8909 only) by entering lowercase base designators in an RNA protocol

More complex modifications require a Workstation to perform protocol editing.

To modify a sequence position, use the arrows to select the base and press the **mod** key (see Figure 3-7). Pressing the **mod** key again will return it to the unmodified designation. Table 3-7 indicates the modified and unmodified base designations.

***NOTE**: Lowercase base designators synthesize different residues and may draw reagent from different reservoirs than uppercase base designators. See "Upper case and lowercase base designators" on page 3-10.*

Table 3-7 Modified and Unmodified Base Positions

Unmodified	Modified
A	a
C	c
G	g
T	t
U	t
5	X
6-9	No change
Mixed sites	No change

Extended Coupling Cycles

All the standard protocols have extended coupling cycles which are accessed by modifying the monomer 5 designation to X. This cycle is useful for adding biotin, linkers, or markers which may require longer coupling times. The X cycle will deliver from reservoir 5 with a coupling time which depends on the selected chemistry (see Table 3-8).

Table 3-8 Chemistry Specific Coupling Times

Chemistry	Standard Coupling Time (min)	Extended Coupling Time (min)
DNA	1.6	15
RNA	13	30
THIO	1.6	15

Base Specific Thioation of DNA

You can synthesize DNA with a mixed phosphate and phosphorothioate backbone using the modified (lowercase) base designations.

This option is available only on the 8909 because the Auxiliary bottle is required for the thioating reagent. The standard oxidizer is placed in the Ox position.

Modified (lowercase) bases in DNA protocols yield a phosphorothioated position. Modified (lowercase) bases in the THIO protocols yield the standard phosphate backbone (see Table 3-9.)

Select the chemistry and protocol that represents the majority of your sequence (thioated or standard DNA).

Table 3-9 Modified Bases for Base Specific Thioation

Reservoir	Base Designation	Chemistry	
		DNA	Thioate
A	A	DNA cycle	THIO cycle
C	C	DNA cycle	THIO cycle
G	G	DNA cycle	THIO cycle
T	T	DNA cycle	THIO cycle
A	a	THIO cycle	DNA cycle
C	c	THIO cycle	DNA cycle
G	g	THIO cycle	DNA cycle
T	t	THIO cycle	DNA cycle
5–9	5–9	DNA cycle	THIO cycle
N/A	Mixed Sites	DNA cycle	THIO cycle
5	X	Extended DNA cycle	Extended THIO cycle

DNA/RNA Hybrids

You can synthesize DNA/RNA hybrids using the modified using the modified (lowercase) base designations.

This option is only available on the 8909 because the 6 to 9 monomer positions are required for the DNA monomers.

To synthesize hybrids, select **RNA chemistry** in the profile. The modified (lowercase) cycles deliver DNA monomer from the appropriate reservoir (see Table 3-10) and perform a DNA coupling cycle.

Table 3-10 Monomer Reservoir Locations for RNA/DNA Hybrids

Reservoir	Base Designator	Monomer Content
A	A	RNA A
C	C	RNA C
G	G	RNA G
T/U	U	RNA U
5	5	User-determined
6	a	DNA A
7	c	DNA C
8	g	DNA G
9	t	DNA T

Naming a Sequence

When you select Name in the Sequence Editor menu, the screen shown in Figure 3-11 is displayed.

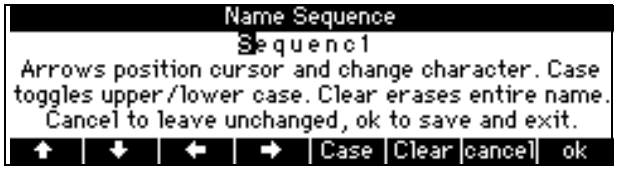


Figure 3-11 Naming a Sequence

The functions that are assigned to the soft keys to enable you to name a sequence are described in Table 3-11.

NOTE: *User-generated names may be displayed in upper and lower case and may contain a maximum of eleven characters.*

Table 3-11 Sequence Naming Keys

Key #	Label	Function
1	↑	Increments (A to Z followed by 1 to 9 and space) the value of the character at the cursor.
2	↓	Decrements (9 to 0 followed by Z to A) the value of the character at the cursor.
3	←	Moves the cursor one position to the left.
4	→	Moves the cursor one position to the right.
5	Case	Toggles the current character between upper and lower case.
6	Clear	Erases the entire sequence name.
7	Cancel	Returns you to the previous screen without saving the new name.
8	OK	Saves the new name and returns you to the previous screen.

Molecular Weight and OD Calculation

Because there is no molecular weight assigned to the numbered monomer reservoirs, these reservoirs are ignored when estimating the molecular weight.

The OD (optical density unit) calculation which is based on a 1 μmol scale assumes:

- 1 OD per 33 mg of product
- 98% coupling efficiency at each synthesis cycle

The OD is calculated as follows:

$$OD = \frac{MW}{33} * (0.98)^{n-1}$$

Where n = sequence length

Melting Point Calculation

For a number of applications using synthetic DNA, it is useful to know the melting temperature (T_M) of the DNA fragment. For example, in order to maximize the specificity of a polymerase chain reaction (PCR), it is necessary to perform the reaction at an optimum annealing temperature. (An approximation of the annealing temperature is 5 to 10 °C below the melting temperature of a primer).

Depending upon the sequence length and type, one of the following calculations is used to determine an approximate melting point for the oligomer.

- Sequences less than 18 bases in length:

$T_m = (4 \times N_{GC}) + (2 \times N_{AT})$

Where: N_{GC} is the total number of C or G bases

N_{AT} is the total number of A or T bases

- Longer DNA sequences:

$T_m = 81.5 + 16.6 \times \text{Log}_{10}[Na+] + 0.41 \times P_{GC} - 600/N$

Where: P_{GC} is the total percentage of C or G bases

N is the sequence length

$[Na^+]$ is 0.1 M

NOTE: *The above calculation may not be reliable for sequences longer than 70 bases, thioated DNA or RNA.*

3.3.2 Running a Sequence

Use the Run option in the Sequence menu to start a synthesis. To start a synthesis:

1. In the Sequence menu, Select **Run**.

   The Sequence selection screen is displayed (see Figure 3-4).

2. Select the Sequence (1 to 63) to be run.

   The column selection screen is displayed (see Figure 3-12).

NOTE: *If you are currently running a synthesis, the column selection screen is not displayed. The available column is selected automatically.*

---

3. Select the Column to be used.

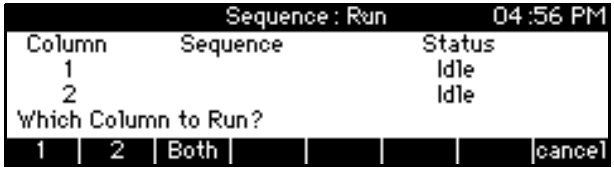


Figure 3-12 Column Selection

NOTE: *You may start the same synthesis on both columns. However, if you wish to run different synthesis protocols on column one and column two, you must repeat this procedure to select and start the second synthesis.*

The run parameters are displayed (see Figure 3-13).

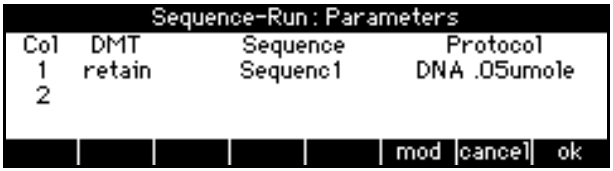


Figure 3-13 Run Parameters

4. To change the parameters, select **Mod**.
5. Press **ok** to save the new parameters and continue.
6. When prompted, load the appropriate column and press **ok** to continue.
7. The **Main menu** is displayed.

   Press **Start** to begin the synthesis.

Modifying Run Parameters

When you select Mod, the screen shown in Figure 3-14 is displayed.

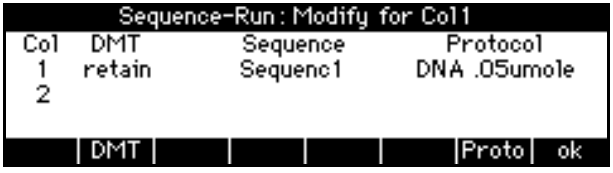


Figure 3-14 Modifying the Run Parameters

The functions described in Table 3-12 are assigned to the soft keys to enable you to modify the run parameters for the current synthesis.

Table 3-12 Modify Run Parameter Keys

Key #	Key Label	Function
2	DMT	Specifies removal or retention of the 5' DMT protecting group.
7	Proto	Displays the available protocols so that you can select the desired synthesis protocol for the selected chemistry. ***NOTE:*** *Select your chemistry in the User Profile. See Section 3.6.6, Specifying a User Profile for more details.*
8	ok	Saves the new parameters and returns you to the previous screen.

3.3.3 Aborting a Synthesis

If, for any reason, you need to abandon a synthesis, use the Abort option to terminate the synthesis after the instrument has been halted. To abort a synthesis:

NOTE: *It is preferable to use the Hold facility to stop the synthesis at the end of a cycle before aborting. Holding will leave the system in a washed condition.*

1. Halt the instrument (see "Holding a Synthesis At End Of Cycle" on page 3-40).
2. Press **Exit** until the Main menu is displayed.
3. Select **Seq** on the Main menu.

4. Select **Abort** on the Sequence menu. The screen shown in Figure 3-15 is displayed.

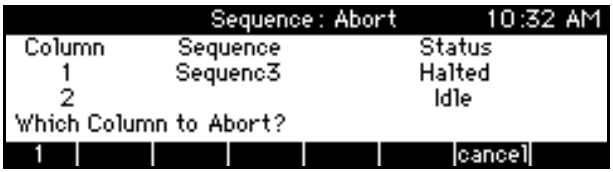


Figure 3-15 Aborting a Synthesis

Dependent on the number of columns that were active, the functions described in Table 3-13 are assigned to the soft keys.

Table 3-13 Keys for Aborting a Sequence

Key #	Label	Function
1	1	Abort the synthesis on Column 1.
2	2	Abort the synthesis on Column 2.
3	Both	Abort both syntheses.
7	Cancel	Return to the Main menu without aborting the synthesis.

5. Select the column number (1,2, or both) on which the synthesis is to be aborted.

3.3.4 Printing a Sequence

Use the Print option to print a copy of selected sequence along with some statistics.

To Print a sequence:

1. In the Sequence Menu, select **Print**.

   The sequence selection menu, shown in Figure 3-4, is displayed.

2. Select the sequence (1 to 63) to be printed.

   A typical sequence printout for a Model 8909 instrument is shown in Figure 3-16.

```
PerSeptive Biosystems
8909 Expedite(TM) Nucleic Acid Synthesis System

Sequence Report
October 13, 1994 02:00:37 PM Instrument: Expedite
_____________________________________________________

SEQUENCE 1, Name = 'AT 28'

5'-AGC-G5G-T8C-G77-AAG-TTC-CAA-TGc-ggt-T-3'-

Base              Frequency
Adenine              5
Cytosine             4
Guanine              6
Thymine              5
Other                8
                    ____
Total=              28

Molecular Weight = 6157.092 g/mole

Purine : Pyrimidine = 11:9 = 1.22

Estimated Optical Density = 108.1 OD's for 1 umole scale


Estimated Melting Temperature = 88.6 C (.1M [Na+])
```

Figure 3-16 Typical Sequence Printout

NOTE: *Because there are no constants assigned to the numbered monomer or lower case positions, these positions are ignored when calculating the molecular weight, purine/pyrimide ratio, optical density and temperature. The optional Expedite 8900 workstation allows you to assign molecular weights to these positions.*

3.3.5 Viewing a Sequence

Use the View option to view the currently stored sequences. The View facility is a read-only tool that allows you to scan the sequence that is currently submitted for synthesis and stored sequences without running the risk of accidentally modifying a sequence.

To view a sequence:

1. In the Sequence menu, select **View**.

   The sequence selection menu (Figure 3-4) is displayed.

2. Select the first sequence to be viewed.

   The selected sequence is then displayed (see Figure 3-17).

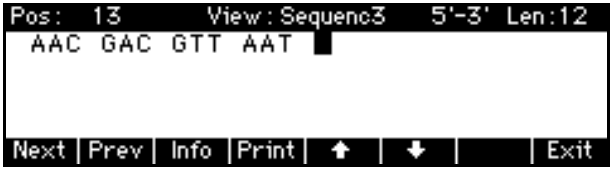


Figure 3-17 Viewing a Sequence

3. Use the keys described in Table 3-14 to view the stored sequences.

View Sequence Key Assignments

The functions that are assigned to the soft keys in the Sequence View facility are described in Table 3-14.

Table 3-14 Sequence Viewing Keys

Key #	Key Label	Function
1	Next	Displays the next stored sequence.
2	Prev	Displays the previously stored sequence.
3	Info	Displays the following summary information associated with the stored sequence. • Sequence number and name • Sequence length • Molecular weight and the calculated OD yield (1 μmol scale) • Purine and pyrimidine ratio • Date and time when the sequence was last saved • Melting temperature (estimated) In this screen, the first and second soft keys are active so that you can scroll through the information about all the stored sequences. Use the Exit key to return to the previous screen.
4	Print	Prints the currently displayed sequence including the associated summary information to an attached printer.
5	↑	If the sequence is not all displayed, scrolls the display up one line.
6	↓	If the sequence is not all displayed, scrolls the display down one line.
8	Exit	Return to the Sequence menu.

3.3.6 Copying a Sequence

Use the Copy option to duplicate a stored sequence.

To copy a sequence:

1. In the Sequence menu, select **Copy**.

   The sequence selection menu is displayed.

2. Select the sequence (1 to 63) you wish to copy.

3. Select the location (sequence name) into which the sequence is to be copied.

   A message similar to the one shown in Figure 3-18 is displayed to allow you to confirm your selection.

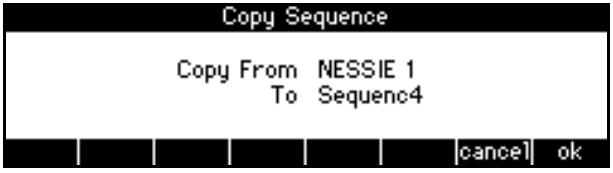


Figure 3-18 Copy Confirmation Screen

4. Complete the procedure by:

   - Pressing **ok** to complete the copy procedure.
   - Pressing **cancel** to abort the process.

5. If desired, rename the copied sequence (see "Naming a Sequence" on page 3-23).

3.4 Status Menu

Use the Status menu to monitor the instrument while a synthesis is in progress. You may use the status option to:

- Get an overview of what is happening with the system.
- Get information about the sequence running on a specific column.
- View the current reagent resources.
- View the reagents needed to complete the synthesis.
- Hold a synthesis.
- Interrupt the synthesis at the end of the current cycle.

When you select Stat from the Main menu, the screen shown in Figure 3-19 is displayed. This is the combined display which gives you an overview of the instrument status.

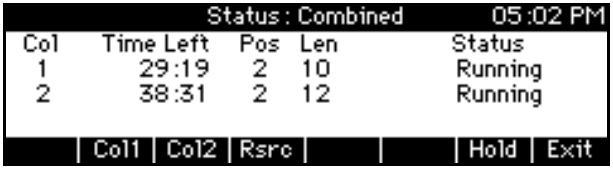


Figure 3-19 Status Mode—Combined Display

3.4.1 Displaying System Information

The following information is displayed on the screen in combined display mode:

- The time left in each synthesis
- The position of the base currently being added in each synthesis
- The length of each sequence
- The current status of the synthesis

Combined Display Mode Key Assignments

The functions that are assigned to the soft keys in combined display mode are described in Table 3-15.

Table 3-15 Combined System Status Key

Key #	Label	Function
2	Col 1	Displays detailed information about the synthesis running on Column 1.
3	Col 2	Displays detailed information about the synthesis running on Column 2.
4	Rsrc	Displays the current reagent resources and requirements (see Figure 3-21) and allows you to set a hold point.
7	Hold/ No Hold	A toggle switch that pauses the syntheses on both columns at the end of the current cycle. The syntheses pauses until you select **No Hold** to resume the syntheses.
8	Exit	To return to the Main menu.

Single Column Display Mode

To display the status for a single column:

Select either the Column 1 or Column 2 soft key.

If you select Column 1, the screen shown in Figure 3-20 is displayed.

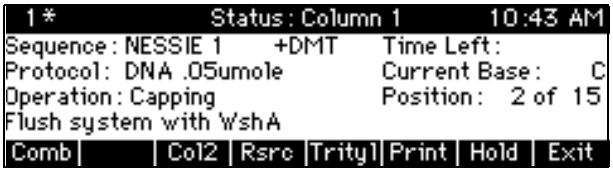


Figure 3-20 Status Mode—Column 1 Display

The following information is displayed for the selected column:

- The sequence name
- The removal or retention of the final 5' DMT (+DMT - retains the 5' DMT)
- Time left in the synthesis
- The protocol being used
- The base currently being added
- The current synthesis subcycle (Deblocking, Coupling, Capping, Oxidizing)
- The length of the oligomer and the position of the current base

Single Column Display Mode Key Assignments

The functions that are assigned to the soft keys in the single column display screens are described in Table 3-16.

Table 3-16 Single Column Display Mode Keys

Key #	Label	Function
1	Comb	To display the Combined synthesis status screen.
2	Col1	Only available if Column 2 is currently displayed. To display the single column display for Column 1.
3	Col 2	Only available if Column1 is currently displayed. To display the single column display for Column 2.
4	Rsrc	To display the amount of reagents required for the current synthesis and amount currently available. This key has the same function in the combined display mode.
5	Trityl	To display the trityl data for the synthesis.
7	Hold/ No Hold	A toggle switch that halts the synthesis on the selected column at the end of the current cycle. The synthesis is halted until you select **No Hold** to resume the synthesis.
8	Exit	To return to the Main menu.

Viewing the System Resources

During a synthesis, the system monitors the reagent usage. The volume of reagents currently in the reservoirs and the volume required to complete the synthesis are displayed in the System Resources screen.

CAUTION

Changing reagent bottles during a synthesis is not recommended. Check the reagent resources prior to initiating a synthesis to make sure that there is a sufficient supply.

If you must replenish reagents during a synthesis, the procedure is described in Section 2.6.1, Replenishing Bottles During a Synthesis.

When you select Rsrc from the Status menu, the screen shown in Figure 3-21 is displayed.

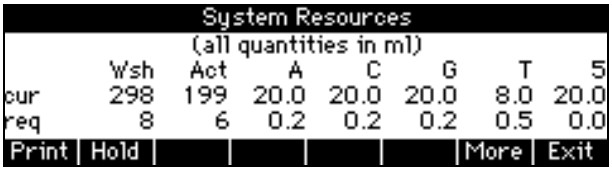


Figure 3-21 Viewing the System Resources

The following information is displayed for each reagent reservoir:

- The amount of reagent currently estimated to be left in the reservoir (cur)
- The amount of reagent required to complete the current syntheses (req)

CAUTION

For an accurate estimate of the current reagent resources, you must reset the bottle configuration each time a reagent reservoir is replenished. See Section 3.6.2, Bottle Change Tool.

Resources Key Assignments

The functions that are assigned to the soft keys in the Resources facility are described in Table 3-17.

Table 3-17 Resources Facility Keys

Key #	Label	Function
1	Print	To print the resource information for the current synthesis including current system resources and requirements.
2	Hold	To set a point in the synthesis where the instrument is stopped temporarily to enable you to replenish the reagent reservoirs.
7	More	To view the next screen of reagent resources.
8	Exit	To return to the previous screen.

3.4.2 Interrupting a Synthesis

You may use the following to interrupt a synthesis:

- Instrument Stop Key
- Stop key in the Main menu
- Hold key in the Status menu

Instrument Stop Key

You may press the instrument Stop key, (see Figure 3-22) at any time during a synthesis. When you press this button, all synthesis operations are halted immediately and remain halted until you press the **Start** key in the Main menu.

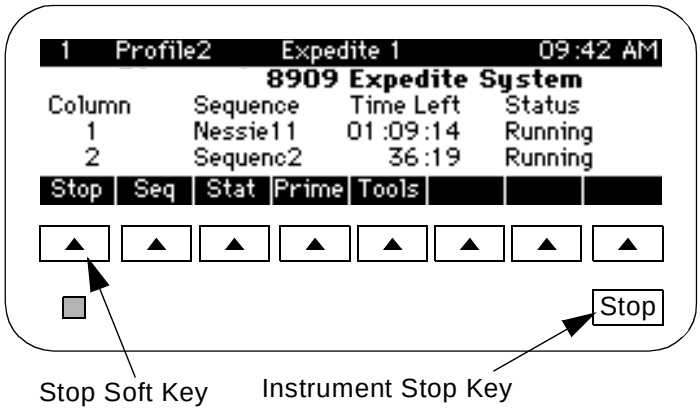


Figure 3-22 Stopping a Synthesis

In the event of an emergency shutdown, the Instrument Stop Key is always available. The software defined Stop key is only available when the Main menu is displayed.

CAUTION

Pausing a synthesis for extended periods in the middle of a cycle may be detrimental to the synthesis. If you use the Stop key, resume the synthesis as soon as possible. To replenish reagents, use the Hold option to pause the synthesis at a safe point between cycles.

Main Menu Stop Option

You may use the software defined Stop key to halt the instrument during a synthesis. The function of this key is the same as the instrument Stop key. However, this key is only displayed on the Main menu when a synthesis has been started and is running.

When you select Stop, synthesis on both columns is halted immediately and held until you press Start to resume the synthesis or use the Abort option in the Sequence menu to cancel the synthesis.

Holding a Synthesis At End Of Cycle

You may use the Hold option in the Status menu to pause a synthesis on one or both columns at the end of the current cycle.

To hold a synthesis at the end of the current cycle:

1. Select **Status** from the Main Menu.

   The combined column screen is displayed (Figure 3-19).

2. Select one of the following options to halt a synthesis:

To halt:	Do the following:
Both columns	Press **Hold** in the combined display screen
Column 1	1. Press **Col 1**. 2. Press **Hold**.
Column 2.	1. Press **Col 2**. 2. Press **Hold.**

When you select Hold, the soft key assignment changes to No Hold (see Figure 3-23) and the synthesis is halted at the end of the current cycle at a chemistry safe point.

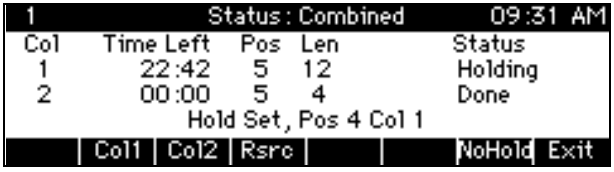


Figure 3-23 Holding a Synthesis

3. To resume the synthesis, select **No Hold**.

   The system recalculates the reagent requirements and resumes the synthesis.

Setting a Hold Point

You may use the Hold option to set a hold at a future point in the synthesis to replenish reagents.

To set a hold point:

1. Select **Status** from the Main menu.
2. Select **Rsrc** from the combined status display screen.

   The system resources screen shown in Figure 3-24 is displayed.

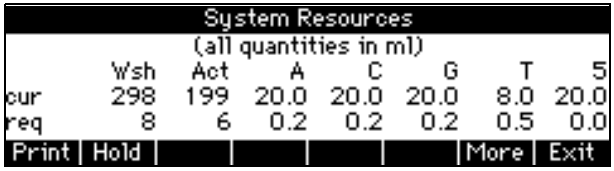


Figure 3-24 System Resources Screen

3. Select **Hold**.

   The screen shown in Figure 3-25 is displayed.

Figure 3-25 Setting a Hold Point

4. Use the arrow keys under the desired column to specify the base addition cycle after which the synthesis is to be paused.
5. Press **ok** to set the hold point.
6. Press **Exit** in the resources screen to return to the combined status display screen.

The hold point is displayed on the screen (see Figure 3-26) and the synthesis continues until the beginning of the cycle at which the hold is set and then halts until you restart it.

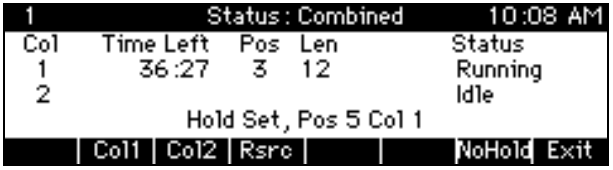


Figure 3-26 Combined Display with Hold Set

7. To restart the synthesis, press **No Hold** on the status menu.

3.5 Prime Menu

Use the Prime menu to:

- Prepare the instrument for synthesis
- Prepare the instrument for storage
- Perform post-synthesis operations

CAUTION

Install a union in place of each reaction column before running any priming routine (except Final Deblock). The priming routines deliver all the reagents through the column to waste, and may cause irreversible damage to the column.

When you select Prime from the Main menu, the screen shown in Figure 3-27 is displayed.

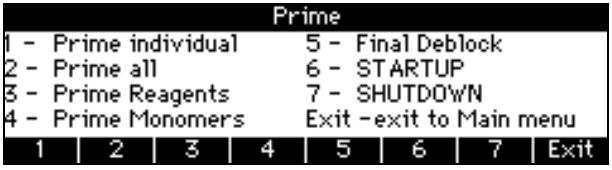


Figure 3-27 Prime Menu

The functions that are assigned to the soft keys in the Prime menu are described in Table 3-18.

Table 3-18 Prime Menu Keys

Key #	Label	Function
1	1	Prime individual reagent passages. See Section 3.5.1, Prime Individual.
2	2	Prime all reagent passages. See Section 3.5.2, Prime All.
3	3	Prime ancillary reagent passages. See Section 3.5.3, Prime Reagents.
4	4	Prime monomer passages. See Section 3.5.4, Prime Monomers.
5	5	Remove the 5' DMT group. See Section 3.5.5, Final Deblock.
6	6	Run the STARTUP routine. See Section 3.5.6, Startup.
7	7	Run the SHUTDOWN routine. Section 3.5.7, Shutdown.
8	Exit	Return to the Main menu.

Column Selection

The priming functions are column-specific. Thus when any option on the Prime menu is selected, the screen shown in Figure 3-28 is displayed to enable you to select the column to be primed.

***NOTE**: Always prime both columns 3 times before beginning a synthesis.*

Figure 3-28 Column Selection

The functions that are assigned to the soft keys are described in Table 3-19.

Table 3-19 Column Selection for Priming

Key #	Label	Function
1	1	Select prime functions for Column 1.
2	2	Select prime functions for Column 2.
8	cancel	Return to the Prime menu.

Table 3-20 Prime Menu Routines

Button	Routine	Description
1	Prime Individual	Primes one reagent position. Pulses the specified reagent 20 times through selected column position.
2	Prime All	Primes all reagent positions: • Prompts user to remove column and replace with a union before starting routine. • Pulses Aux, Ox, Caps, Act 20 times each; Pulses monomers 10 times each; Pulses Dblk 120 times, Wsh A 150 times, and Wsh 30 times. • Dries the selected column position(s) for 10 seconds. • Beeps 10 times at completion of routine.
3	Prime Reagents	Primes everything but monomer positions. Same as Prime All, except the monomer positions are not primed.
4	Prime Monomers	Primes only monomer positions. Same as Prime All, except Aux, Ox, Caps, Dblk, and Wsh A (A train reagents) are *not* primed.
5	Final Deblock	Performs final deblock and wash: • Performs a final deblock cycle for the scale of the completed synthesis, which is the same as in the standard 0.05, 0.2, and 1 µmole protocols. If the synthesis is aborted, a default final deblock cycle is performed. • Washes the column with 300 pulses of Wsh A. • Dries the selected column position for 60 seconds. • Beeps 10 times at the completion of routine.

Table 3-20 Prime Menu Routines (Continued)

Button	Routine	Description
6	STARTUP	Prepares an instrument for synthesis from a shutdown state: • Prompts user to fill bottles with ACN and put a union in both column positions. • Performs a “prime all” on each position, 3 times on each column. • Prompts user to put fresh ACN in B train bottles (monomers, Act, and Wsh). • Performs a “prime all” on each position, 3 times on each column. • Prompts user to install reagents. • Performs a “prime all” on each position, 3 times on each column. • Dries the selected column position(s) for 10 seconds. • Beeps 10 times at completion of routine.

Table 3-20 Prime Menu Routines (Continued)

Button	Routine	Description
7	SHUTDOWN	Prepares an instrument for a short-or long-term shutdown: • Prompts user to put a union in both column positions. • Washes each train, column position, and waste line with 300 pulses of ACN (4.5 mL). • Dries each train, column position and waste line with gas for 60 seconds. • Prompts user to press cancel to end routine (i.e. if it is a short-term shutdown) or ok to continue with long-term shutdown. • If ok is chosen: • Prompts user to fill all bottles with ACN. • Flows ACN from each bottle through both column positions. • Pulses ACN from each bottle through both column positions. • Flows ACN from each bottle through both column positions. • Prompts user to put empty bottles on instrument. • Flows gas from each position through both column positions. • Pulses gas from each position through both column positions. • Flows gas from each position through both column positions. • Beeps 10 times at completion of routine.

3.5.1 Prime Individual

***NOTE**: Always prime both columns 3 times before beginning a synthesis.*

Use the Prime Individual facility to manually prime each fluid passage. Prime individual is most commonly used when changing an individual reagent bottle. When you select Prime Individual and choose the column to prime, the screen shown in Figure 3-29 is displayed.

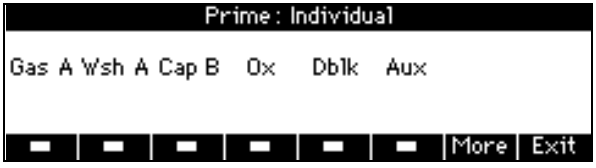


Figure 3-29 Prime Individual—First Screen

The individual reagents are displayed (press More to bring up a second and third screen with additional reagent selections). The functions assigned to the soft keys are described in Table 3-21.

The procedure for priming an individual fluid passage is as follows:

1. Press the soft key associated with the desired reagent to begin the flow of reagent from the reservoir.

   The reagent injector is activated for one priming cycle to supply 20 pulses (~320 µL) of reagent to the fluid passages. Key #3 is available to enable you to stop the process at any time (see Figure 3-30).

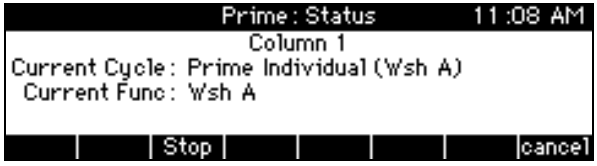


Figure 3-30 Prime Individual Pulse/Cycle Screen

Table 3-21 Prime Individual Keys

Key #	Name	Function
First Screen Keys		
1	Gas A	Blows gas through the A-train to the column.
2	Wash A	Delivers reagent from the external Wash A reservoir position (8909).
3	Caps	Delivers reagent from the Cap A and Cap B reservoir positions.
4	Ox	Delivers reagent from the Oxidizer reservoir position.
5	Dblk	Delivers reagent from the Deblock reservoir position and is diverted to chlorinated waste.
6	Aux	Delivers reagent from the Aux reservoir position (8909).
7	More (All Screens)	Displays the next screen with additional reagent reservoir positions.
8	Exit (All Screens)	Return to the Prime menu.
Second Screen Keys		
1	Wash	Delivers reagent from the Wash reservoir position.
2	Act	Delivers reagent from the Activator reservoir position.
3	A	Delivers reagent from Adenosine monomer reservoir position.

Table 3-21 Prime Individual Keys (Continued)

Key #	Name	Function
4	C	Delivers reagent from the Cytidine monomer reservoir position.
5	G	Delivers reagent from the Guanosine monomer reservoir position.
6	T/U	Delivers reagent from the Thymidine (Uridine) monomer reservoir position.
Third Screen Keys		
1	5	Delivers reagent from the monomer reservoir 5 position.
2	6	Delivers reagent from the monomer reservoir 6 position (8909).
3	7	Delivers reagent from the monomer reservoir 7 position (8909).
4	8	Delivers reagent from the monomer reservoir 8 position (8909).
5	9	Delivers reagent from the monomer reservoir 9 position (8909).
6	Gas B	Blows gas through the B-train to waste.

2. After one priming cycle, the functions described in Table 3-22 are assigned to the soft keys.

Table 3-22 Priming Keys

Key #	Name	Function
1	Pulse	Delivers a single pulse (~16 µL) of reagent.
2	Cycle	Delivers a single cycle (20 pulses or ~320 µL) of reagent).
8	cancel	Return to the Prime Individual menu.

3. Complete the priming procedure by selecting:

   - Wash A
   - Wash B
   - Gas A
   - Gas B

   This flushes the common passages and prevents cross contamination with other reagents.

NOTE: *When using Prime Individual, it is possible to deliver a small amount of chlorinated waste to the non-chlorinated waste container. If this is a problem, use the pre-programmed priming routines (Prime All, Prime Reagents, Prime Monomers) which wash all the chlorinated waste to the appropriate waste container.*

3.5.2 Prime All

***NOTE**: Always prime both columns 3 times before beginning a synthesis.*

Use the Prime All (Monomers and Reagents) routine to automatically prime each reagent delivery passage. The procedure for priming all the reagent passages is as follows:

1. Select **Prime All**, and select the column.

   The screen shown in Figure 3-31 is displayed. There is a programmed hold until you press **ok**.

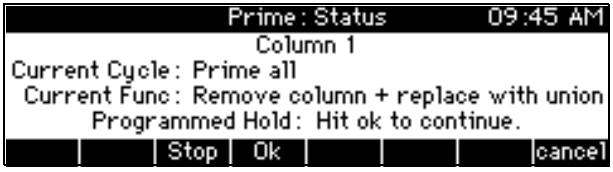


Figure 3-31 Prime All—First Screen

2. Remove the column and replace it with a union.
3. Press **ok**.

   The screen shown in Figure 3-32 is displayed.

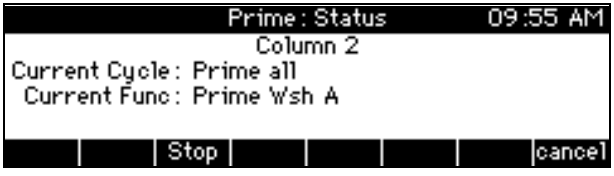


Figure 3-32 Prime All—Second Screen

Reagents are delivered through all the fluid passages sequentially from the following reservoir positions:

- Wash A (30 pulses)
- Cap A and Cap B (20 pulses)
- Wash A (30 pulses)
- Oxidizer (20 pulses)
- Wash A (30 pulses)
- Aux (8909 only—20 pulses)
- Wash A (30 pulses)
- Deblock [diverted to chlorinated waste (120 pulses)]
- Wash A [diverted to chlorinated waste (30 pulses)]
- Activator (20 pulses)
- Each monomer reservoir (10 pulses)
- Wash (30 pulses)

During the priming routine, the Stop key is available and the current function is displayed on the screen.

After all the reagent passages are primed, the common passages are blown out with gas for 10 seconds in preparation for synthesis.

On completion of the priming cycle, the instrument beeps 10 times.

After one priming cycle, the screen shown in Figure 3-33 is displayed and the functions described in Table 3-23 are assigned to the soft keys.

Figure 3-33 Prime All—Third Screen

Table 3-23 Priming Keys—Preprogrammed Routines

Key #	Name	Function
2	Cycle	To repeat the priming routine.
8	cancel	To return to the Prime menu or stop the current priming routine.

3.5.3 Prime Reagents

***NOTE**: Always prime both columns 3 times before beginning a synthesis.*

Use the Prime Reagents routine to automatically prime all reagent delivery passages from the ancillary reagent reservoirs. To prime all the reagent lines:

1. Select **Prime Reagents**, and select the column.

   The screen shown in Figure 3-34 is displayed. There is a programmed hold until you press **ok**.

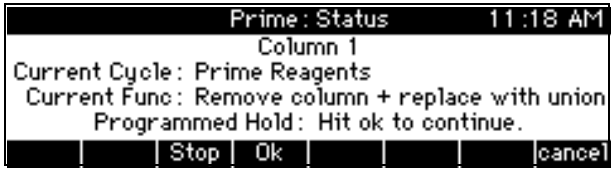


Figure 3-34 Prime Reagents—First Screen

2. Remove the column and replace it with a union.
3. Press **ok**.

   The screen shown in Figure 3-35 is displayed.

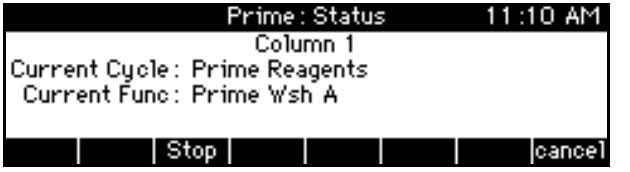


Figure 3-35 Prime Reagents—Second Screen

The reagent passages are primed sequentially from the following reservoir positions:

- Wash A (30 pulses)
- Cap A and Cap B (20 pulses)
- Wash A (30 pulses)
- Oxidizer (20 pulses)
- Wash A (30 pulses)
- Aux (8909 only—20 pulses)
- Wash A (30 pulses)
- Deblock [diverted to chlorinated waste (120 pulses)]
- Wash A [diverted to chlorinated waste (30 pulses)]
- Activator (20 pulses)
- Wash (30 pulses)

During this priming routine, the Stop key is available and the current function is displayed on the screen.

After all the reagent passages are primed, the common passages are blown out with gas for 10 seconds in preparation for synthesis.

On completion of the priming cycle, the instrument beeps 10 times and the functions described in Table 3-23 are assigned to the soft keys. You may press **Cycle** to repeat the priming routine or **cancel** to return to the Prime menu.

3.5.4 Prime Monomers

***NOTE**: Always prime both columns 3 times before beginning a synthesis.*

Use the Prime Monomers routine to automatically prime the monomer solutions and the B-train ancillary reagents delivery passages. The procedure for priming the monomer passages is as follows:

1. Select Prime Monomers, and select the column.

   The screen shown in Figure 3-36 is displayed. There is a programmed hold until you press **Ok**.

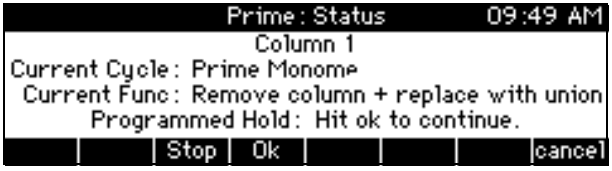


Figure 3-36 Prime Monomers—First Screen

2. Remove the column and replace it with a union.

3. Press **Ok**.

   The screen shown in Figure 3-37 is displayed.

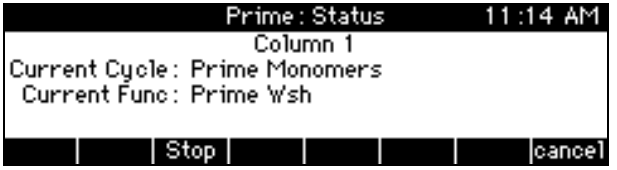


Figure 3-37 Prime Monomers—Second Screen

The reagent passages are primed sequentially from the following reservoir positions:

- Activator (20 pulses)
- Each monomer reservoir (10 pulses)
- Wash (30 pulses)

During the priming routine, the Stop key is available and the current function is displayed on the screen.

After all the reagent passages are primed, the common passages are blown out with gas for 10 seconds in preparation for synthesis.

On completion of the priming cycle, the instrument beeps 10 times and the functions described in Table 3-23 are assigned to the soft keys. You may press **Cycle** to repeat the priming routine or **cancel** to return to the Prime menu.

3.5.5 Final Deblock

Use the Final Deblock routine to remove the final DMT group manually. If you did not select automatic removal of the final DMT, you may use this option at the end of the synthesis. When you select Final Deblock and select the desired column, the screen shown in Figure 3-38 is displayed.

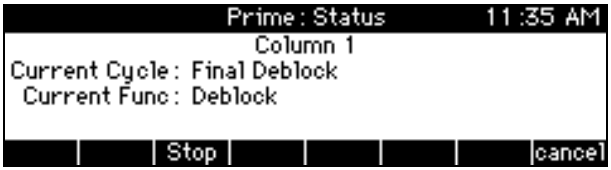


Figure 3-38 Final Deblocking Routine

The system performs a deblock cycle on the selected column. The final deblock routine will use the same deblock routine of the selected protocol on that column.

If there is no synthesis on the column then the default final deblock routine is used (which is the same as that used with the standard 0.05, 0.2 and 1.0 μmole protocols).

During the deblocking routine, the Stop key is available and the current function is displayed on the screen. After the routine has been performed, the functions described in Table 3-23 are assigned to the soft keys and you may press **Cycle** to repeat the deblocking routine or press **cancel** to return to the Prime menu.

3.5.6 Startup

Use the Startup routine to prepare the instrument for synthesis after it has been shut down for a period of time. The procedure for running the Startup routine is as follows:

1. Select **Startup**, and select the column.

   The screen shown in Figure 3-39 is displayed.

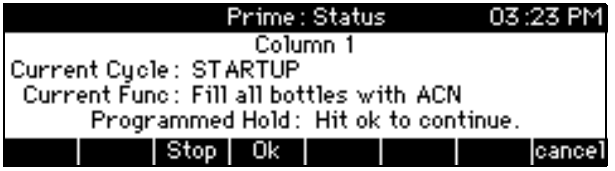


Figure 3-39 Startup Routine—First Screen

2. Fill the reagent reservoirs with acetonitrile (ACN) as follows:

   - Place approximately 5 mL of ACN in the monomer reservoirs.
   - Place 25 mL of ACN in all the remaining reagent reservoirs.

3. Press **Ok** to continue. The screen shown in Figure 3-40 is displayed.

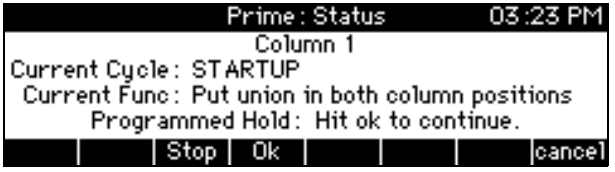


Figure 3-40 Startup Routine—Second Screen

4. Place a union in both column positions and press **Ok**.

   The system primes all the reagent passages three times. During the priming routine, the Stop key is available and the current function is displayed on the screen.

   On completion of the first step of the startup routine, the screen shown in Figure 3-41 is displayed.

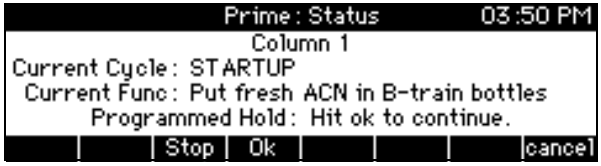


Figure 3-41 Startup Routine—Third Screen

5. Replace the acetonitrile (ACN) in the B-train reservoirs with fresh dry acetonitrile (<.005% H_20).

   - Place approximately 5 mL of ACN in the monomer reservoirs.
   - Place approximately 25 mL of ACN in the Activator (ACT) and Wash (Wash) reservoirs.
   - Press **Ok**.

   The system primes all the reagent passages three times. During the priming routine, the Stop key is available and the current function is displayed on the screen.

On completion of this step of the priming routine, the screen shown in Figure 3-42 is displayed.

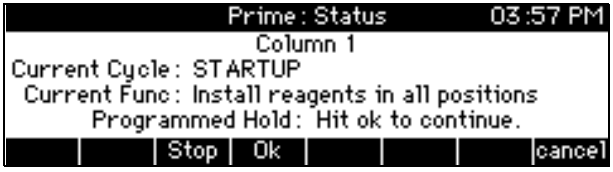


Figure 3-42 Startup Routine—Fourth Screen

6. Install new reagent bottles in all reservoir positions. See Section 2.4.2, Installing the Reagents.

7. Press **Ok**.

   The system primes all the reagent passages three times. During the priming routine, the Stop key is available and the current function is displayed on the screen.

   After all the reagent passages are primed, the common passages are flushed with acetonitrile and passages are blown out with blanket gas for 10 seconds in preparation for synthesis.

   On completion of the routine, the instrument beeps ten times and you are prompted to press **Ok** to exit the routine.

   The functions described in Table 3-23 are assigned to the soft keys. You may press **Cycle** to repeat the priming routine or **cancel** to return to the Prime menu.

3.5.7 Shutdown

Use the Shutdown routine to wash out all the fluid passages in preparation for short or long term storage. Short term shutdown is a period of less than two weeks. Any period of time greater than two weeks is long term shutdown.

The Shutdown routine has two components.

- Short term shutdown is run whenever the instrument is to be shutdown.
- Long term shutdown is a continuation of the short term program which is selected after the short term shutdown routine has been run.

The procedure for running the Shutdown routine is as follows:

1. Select Shutdown, and select the column.

   The screen shown in Figure 3-43 is displayed.

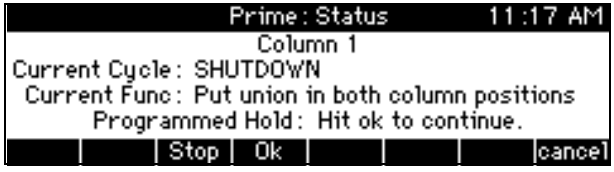


Figure 3-43 Shutdown Routine—First Screen

2. Place a union in both column positions and press **Ok**.

   The system washes each reagent train with acetonitrile from the Wash reservoirs and gas is blown through the reagent passages for 60 seconds to dry them out. On completion of the short term shutdown, the screen shown in Figure 3-44 is displayed.

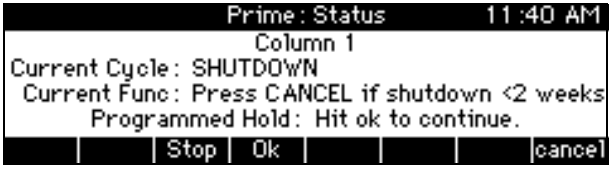


Figure 3-44 Shutdown Routine—Second Screen

NOTE: *If the instrument is to be shut down for less than two weeks, press* ***cancel*** *to exit the shutdown routine. If the instrument is to be shut down for longer than two weeks, press* ***Ok*** *to continue with the long term shutdown.*

The screen shown in Figure 3-45 is displayed.

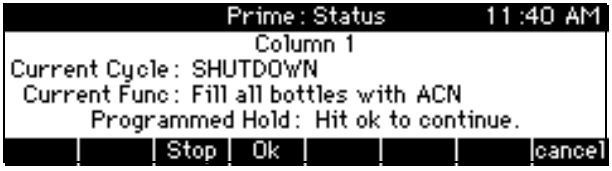


Figure 3-45 Shutdown Routine—Third Screen

3. Fill the reagent reservoirs with acetonitrile (ACN) as follows:

    - Place approximately 10 mL of ACN in the monomer reservoirs.
    - Place approximately 25 mL of ACN in all the remaining reagent reservoirs.

4. Press **Ok** to continue. The system flows acetonitrile through the reagent passages from all the reservoir positions. On completion, the screen shown in Figure 3-46 is displayed.

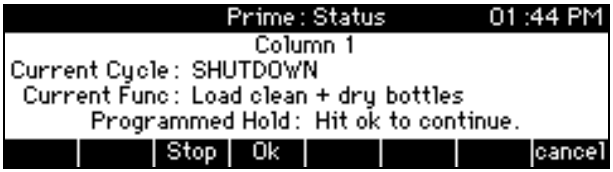


Figure 3-46 Shutdown Routine—Fourth Screen

5. Load a clean dry bottle onto each reservoir position and press **Ok**.

   The system flows gas through the system from each reservoir position to dry out all the reagent passages. On completion of the routine, the instrument beeps ten times and you are prompted to press **Ok** to exit the routine.

   The functions described in Table 3-23 are assigned to the soft keys. You may press **Cycle** to repeat the priming routine or **cancel** to return to the Prime menu.

3.6 Tools Menu

Use the Tools menu to:

- Run diagnostics
- Reset the reagent bottle volumes
- Adjust the display contrast
- Clear or print the history log
- Configure system parameters

When you select Tools from the Main menu, the screen shown in Figure 3-47 is displayed:

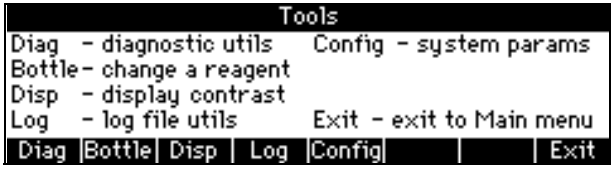


Figure 3-47 Tools Menu

Tools Menu Key Assignments

The functions that are assigned to the soft keys in the Tools menu are described in Table 3-24.

Table 3-24 Tools Menu Keys

Key #	Label	Function
1	Diag	To access the various diagnostic routines which insure proper instrument functionality as well as provide automated trouble shooting. See Section 3.6.1, Diagnostic Routines.
2	Bottle	To reset the reagent bottle contents. See Section 3.6.2, Bottle Change Tool.
3	Disp	To adjust the front panel display (LCD) contrast. See Section 3.6.3, Display Tool.
4	Log	To access the log file maintenance facility. Section 3.6.4, Log Tool.
5	Config	To access the facility for setting system parameters. See Section 3.6.5, Configuration Tool.
8	Exit	To return to the Main menu.

3.6.1 Diagnostic Routines

Use the Diagnostic Routines tool to access various system diagnostic procedures. When you select Diagnostics from the Tools menu, the screen shown in Figure 3-48 is displayed.

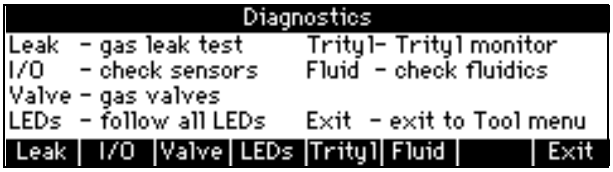


Figure 3-48 Diagnostics Menu

The functions that are assigned to the soft keys in the Diagnostics menu are described in Table 3-25.

Table 3-25 Diagnostic Routines Keys

Key #	Name	Function
1	Leak	To perform the automated gas leak isolation tests.
2	I/O	To check the gas sensors and turn on or off input and output events.
3	Valve	To perform gas valve checkout routines.
4	LEDs	To test the reagent solenoid electronic control circuits.
5	Trityl	To access the Trityl Monitor diagnostic tool.
6	Fluid	To collect and measure the output of the fluidics module.
8	Exit	To return to the Tools menu.

Leak Diagnostics

The Expedite instrument has two gas pressure sensors:

- The high pressure sensor
- The low pressure sensor

The high pressure sensor continuously monitors the input gas pressure. If it detects a pressurization failure (<10 psi), the instrument will halt at the end of the current cycle. You are warned of the situation and you must press the **Ok** key before further operation is allowed. If desired, you may disable this function by modifying the User Profile (see Section 3.6.6, Specifying a User Profile).

The low pressure sensor measures the severity of gas leaks when the instrument is active. These leaks are typically caused by removing bottles or attaching bottles that have defective O-rings. If the low pressure sensor does not detect a minimal pressure (4 psi) for 5 consecutive seconds, the instrument will halt at the end of the current cycle. You are warned of the situation and must respond and eliminate the leak before further operation is allowed. If desired, you may disable this function by modifying the User Profile (see Section 3.6.6, Specifying a User Profile).

The Gas Leak option in the Diagnostics menu tests the integrity of the high and low pressure gas systems. When you select Leak from the Diagnostics menu and press Start, the screen shown in Figure 3-49 is displayed.

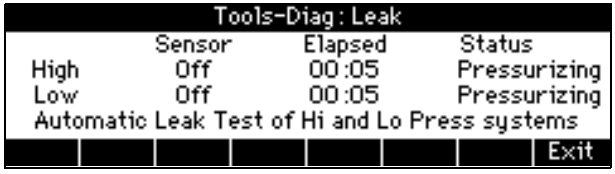


Figure 3-49 Leak Diagnostics

The functions that are assigned to the soft keys are described in Table 3-26.

Table 3-26 Leak Diagnostics Keys

Key #	Name	Function
1	Start	To start the automatic leak test. The leak test may take about 5 minutes to run to completion.
8	Exit	To return to the diagnostics menu.

The Gas Leak Test routine does the following:

1. Opens the high and low pressure inlets for 20 seconds to pressurize the system.
2. Turns off the high and low pressure inlets to isolate the systems from each other and the gas source.
3. Measures the time it takes for the pressure to drop low enough to trigger the gas sensors.

   The system passes if the high pressure is maintained for >5 seconds and the low pressure is maintained for >3 minutes.
4. If the time is less than the defined value, the system reports a failure.

Gas Saver

The Gas Saver, which is activated by default, reduces overall gas consumption. If desired, you may disable Gas Saver in the User Profile (see Section 3.6.6, Specifying a User Profile).

When the gas saver is enabled, the system periodically performs the Gas Leak Test routine. If the system fails the test, a warning is issued and the gas is shut off until the instrument is activated and the gas saver test fail signal is acknowledged (see Figure 3-50).

The instrument is considered to be idle if there has been no keyboard or reagent delivery activity for 5 minutes. The Gas Saver is activated and remains operational until the instrument is activated (any key is pressed).

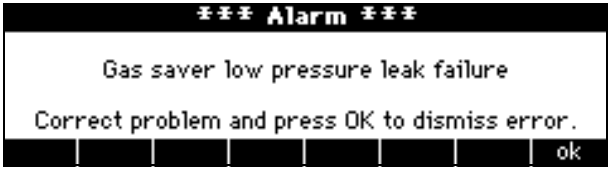


Figure 3-50 Gas Saver Pressure Failure Warning

The Startup Leak Test automatically runs a shortened version of the gas leak diagnostic test when you press Start to begin or continue a synthesis. If the test fails, a warning is issued and the instrument is halted at the end of the cycle. The Startup Leak Test may be enabled or disabled in the User Profile.

Sensor Check

The Sensor check option monitors the input sensors. When you select I/O, the system checks to see if the sensors are activated and reports the status in the screen shown in Figure 3-51.

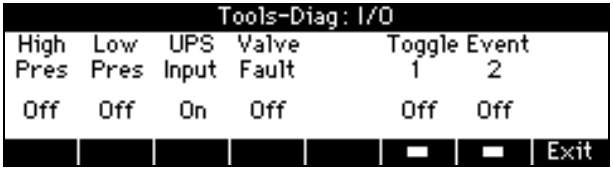


Figure 3-51 Gas Sensor Diagnostics

In this screen, soft keys six and seven are active to enable you to turn on or off the external contact closures for testing purposes.

Valve test

The Valve option allows you to toggle on and off the high and low pressure gas manifold valves and waste divert valves.

This screen also displays the total number of pulses for the instrument. The number of pulses increments up to 16,777,216, then resets to zero and begins incrementing again.

When you select Valve, the screen shown in Figure 3-52 is displayed.

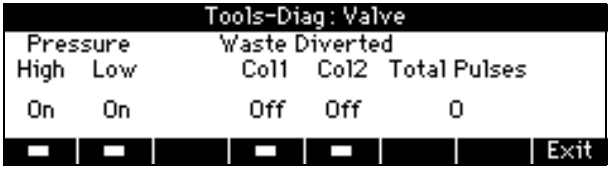


Figure 3-52 Valves Diagnostics With Trityl Monitor Installed

The functions that are assigned to the soft keys are described in Table 3-27.

Table 3-27 Valve Test Keys

Key #	Name	Function
1	-	Turn on or off the high pressure gas valve.
2	-	Turn on or off the low pressure gas valve.
4	-	Turn on or off the Col 1 waste divert valve.
5	-	Turn on or off the Col 2 waste divert valve.
8	Exit	To return to the Diagnostics menu.

LED Test

Use the LED (Light Emitting Diode) test to exercise the fluid control solenoids.

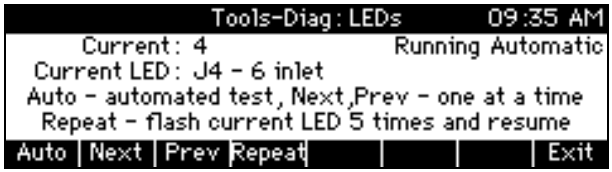


Figure 3-53 LED Test Facility

When a solenoid is activated, you will hear a click and the appropriate LED on the driver board (inside the instrument cabinet) lights up. (The LEDs are only accessible to service personnel.)

The LED test allows testing of the valves manually or automatically. The functions that are assigned to the soft keys are described in Table 3-28.

Table 3-28 LED Test Keys

Key #	Name	Function
1	Auto	Run the automated test to actuate each solenoid valve sequentially.
2	Next	Manually activate the next solenoid valve.
3	Prev	Manually activate the previous solenoid valve.
4	Repeat	Manually activate the current solenoid five times.
8	Exit	To return to the Diagnostics menu.

Trityl This screen displays the value of the output signal from the monitor for each column.

Fluid Test The Fluid test allows you to assess the performance of the fluidic system by collecting and measuring the volumetric output from selected fluidic pathways.

See Section 4.3, Flow and Volume Test, for the procedure to run this test.

3.6.2 Bottle Change Tool

When replenishing reagents, use the Bottle Change Tool to:

- View the reagent resources
- Reset the reagent bottle volume
- Turn off the reservoir blanket gas

1. In the Tools menu, select **Bottle**.

   The screen shown in Figure 3-54 is displayed.

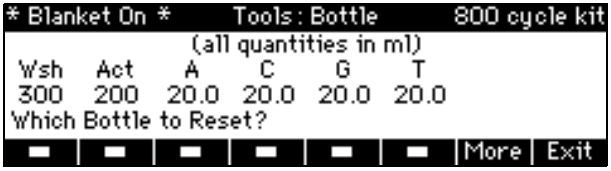


Figure 3-54 Bottle Change Tool—First Screen

   The reagents (B-train), the reagent kit size (800, 400, or 150 cycles), and the current volume in each bottle are displayed on the screen. These reagents must be kept moisture free, therefore the blanket gas is not suspended.

2. Change the reagent bottles (see Section 2.4.2, Installing the Reagents).

3. Press the appropriate keys to reset the volumes of the bottles that have been changed or refilled.

NOTE: *You may press the same key a second time to recall the volume the system believes is currently left in the bottle.*

4. Press **More** to view the next screen, shown in Figure 3-55.

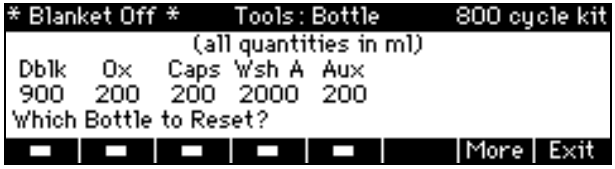


Figure 3-55 Bottle Change Tool—Second Screen

5. To minimize the release of noxious vapors, the blanket gas is automatically suspended while the A-train reagents (Oxidizer, Deblock, Cap A and Cap B) are being installed. During blanket suspension, the Gas Saver and all alarms are also disabled. The monomer reservoirs are blanketed continuously.
6. Change the reagent bottles.
7. Press the appropriate keys to reset the bottles that have been changed or refilled.
8. Press **Exit** to return to the Tools menu. Blanketing will be re-activated.

NOTE: *You cannot recall the previous value after you exit this screen and return to the Tools menu.*

The functions that are assigned to soft keys in the bottle tool screens and the capacity of each reagent reservoir, according to reagent kit size, are listed in Table 3-29.

Table 3-29 Bottle Change Tool Keys

Key #	Reservoir Name or Key Function	Reservoir Capacity					
		Model 8909 # of Cycles			Model 8905 # of Cycles		
		800	400	150	800	400	150
First Screen Keys							
1	Wash	300 mL		N/A	N/A		450 mL
2	Act	200 mL		N/A	N/A		60 mL
3	A	20 mL	10 mL	N/A	N/A		5 mL
4	C	20 mL	10 mL	N/A	N/A		5 mL
5	G	20 mL	10 mL	N/A	N/A		5 mL
6	T	20 mL	10 mL	N/A	N/A		5 mL
7	More—Go to next screen	N/A					
8	Exit to tools manu	N/A					
Second Screen Keys							
1	Dblk	900 mL		N/A	N/A		180 mL
2	Ox	200 mL		N/A	N/A		60 mL
3	Caps	200 mL		N/A	N/A		60 mL
4	Wash A	2000 mL		N/A	N/A		
5	Aux	200 mL		N/A	N/A		N/A

Table 3-29 Bottle Change Tool Keys (Continued)

Key #	Reservoir Name or Key Function	Reservoir Capacity					
		Model 8909 # of Cycles			Model 8905 # of Cycles		
		800	400	150	800	400	150
Third Screen Keys							
1	5 Monomer reservoir	20 mL	10 mL	N/A	N/A		
2	6 Monomer reservoir	20 mL	10 mL	N/A	N/A		
3	7 Monomer reservoir	20 mL	10 mL	N/A	N/A		
4	8 Monomer reservoir	20 mL	10 mL	N/A	N/A		
5	9 Monomer reservoir	20 mL	10 mL	N/A	N/A		

Automatic Deblanket

The automatic deblanket turns off the flow of gas blanketing the reagent bottles when the bottle change tool is used to replenish the A-train reagents (the screen shown in Figure 3-55 is displayed). This minimizes the release of noxious vapors during reagent replenishment.

If desired, you can disable this automatic shutoff by turning off the Automatic Deblanket option in the User Profile (see Section 3.6.6, Specifying a User Profile).

3.6.3 Display Tool

Use the Display Tool to adjust the LCD (Liquid Crystal Display) contrast setting for different viewing angles. When you select Display on the Tools menu, the screen shown in Figure 3-56 is displayed.

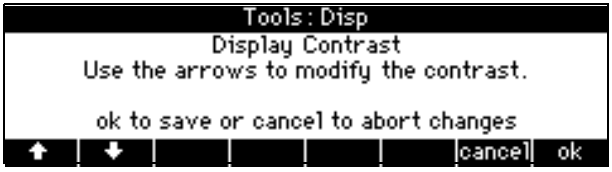


Figure 3-56 Changing the Display Contrast

The functions that are assigned to the soft keys are described in Table 3-30.

Table 3-30 Keys for Changing the Display Contrast

Key #	Label	Function
1	↑	Increase the contrast of the display.
2	↓	Decrease the contrast of the display.
7	cancel	To abort any changes to the display contrast.
8	ok	To save the modified contrast setting and return to the Tools menu.

3.6.4 Log Tool

Use the Log tool to clear the log file or print the log file on an optional printer.

The log file contains a chronological history of the activity of the instrument. The information in a log file includes:

- A record of each synthesis
- The alarms triggered
- Trityl data
- Manual functions activated

A typical log file is shown in Figure 3-57.

```
************************************************************
************************************************************
Expedite(TM) Nucleic Acid Synthesis System
************************************************************
************************************************************

-(  7) 1 05/04/93, 03:09:44 PM -> Sequence Selected
 Instrument Name: ''
 Chemistry Type: 'DNA'
 Sequence Name: 'NESSI 10'
 Sequence: (3'-5')
  'TCC-ATT-GGC-CAA'
 Protocol: 'DNA .05umole'
 Operator: ''
 Remove DMT: 'No'
 Universal Support: 'No'
-(  4) N 05/04/93, 03:09:52 PM -> Instrument Started
-(  7) 2 05/04/93, 03:11:04 PM -> Sequence Selected
 Instrument Name: ''
 Chemistry Type: 'DNA'
 Sequence Name: 'Sequenc2'
 Sequence: (3'-5')
  'AAA-A'
 Protocol: 'DNA .05umole'
 Operator: ''
 Remove DMT: 'Yes'
 Universal Support: 'No'
-( 10) N 05/04/93, 03:11:06 PM -> Low Pressure Gas Error
 Col: '1', Mer: '2', Step: '11', Func: '3'
 Col: '2', Mer: '2', Step: '1', Func: '1'
-( 11) N 05/04/93, 03:11:06 PM -> High Pressure Gas Error
 Col: '1', Mer: '2', Step: '11', Func: '3'
 Col: '2', Mer: '2', Step: '1', Func: '1'
-( 10) N 05/04/93, 03:11:31 PM -> Low Pressure Gas Error
 Col: '1', Mer: '2', Step: '11', Func: '3'
 Col: '2', Mer: '2', Step: '3', Func: '17'
-( 11) N 05/04/93, 03:11:31 PM -> High Pressure Gas Error
 Col: '1', Mer: '2', Step: '11', Func: '3'
```

Figure 3-57 Expedite History Log

When you select Log from the Tools menu, the screen shown in Figure 3-58 is displayed.

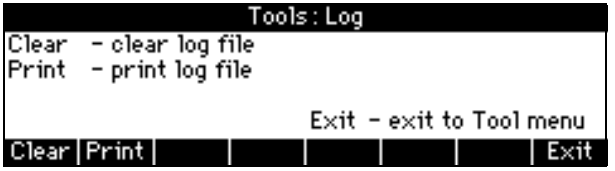


Figure 3-58 The Log Tool

The functions that are assigned to the soft keys are described in Table 3-31.

Table 3-31 Log Tool Keys

Key #	Name	Function
1	Clear	To clear the current system log file on the disk.
2	Print	To print out a hard copy of the current log. A typical log is shown in Figure 3-57.
8	Exit	To return to the Tools menu.

NOTE: *Use caution when clearing the log. You may remove vital information used to diagnose and service the instrument.*

3.6.5 Configuration Tool

The Configuration tool is used to set various system parameters. When you select Configure from the Tools menu, the screen shown in Figure 3-59 is displayed.

Figure 3-59 Configuration Tool

The functions assigned to the soft keys are described in Table 3-32.

Table 3-32 Configuration Tool Keys

Key #	Name	Function
1	Time	To set the instrument clock.
2	Host	To define the address of the instrument for communicating with an Expedite workstation.
3	Profil	To define user specific default parameters. See Section 3.6.6, Specifying a User Profile for details.
4	Ver	To display the current system software version.
5	Name	To enable you to specify a unique name for the instrument.
6	Print	To print the instrument configuration report.
8	Exit	To return to the Tools menu.

Setting the Clock

When you select Time in the Configuration menu, the screen shown in Figure 3-60 is displayed.

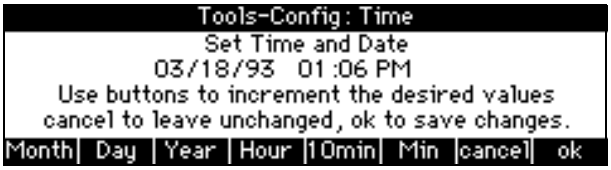


Figure 3-60 Setting the Instrument Clock

The functions described in Table 3-33 are assigned to the soft keys to enable you to reset the instrument's time clock.

Table 3-33 Keys for Setting the Time Clock

Key #	Name	Function
1	Month	To increment the month by one.
2	Day	To increment the day by one.
3	Year	To increment the year by one.
4	Hour	To increment the hour by one.
5	10min	To increment the minutes by ten.
6	Min	To increment the minutes by one.
7	cancel	To return to the Tools Configuration menu without saving changes to the time clock.
8	ok	To save changes to the time clock and return to the Tools Configuration menu.

NOTE: *If the instrument is connected to an Expedite Workstation, the instrument clock is automatically synchronized with the computer clock.*

Setting Host Parameters

Use the Host option to set the instrument address and display the mode of operation between the instrument and the Expedite Workstation. Instruments connected to an Expedite Workstation must have a unique address between 1 and 32. Instruments not connected to an Expedite Workstation have an address of 0, and the mode of operation is autonomous.

When you select Host on the Configuration menu, the screen shown in Figure 3-61 is displayed. To set the instrument address press the key below the address. Each time you press the key, the address is incremented by one.

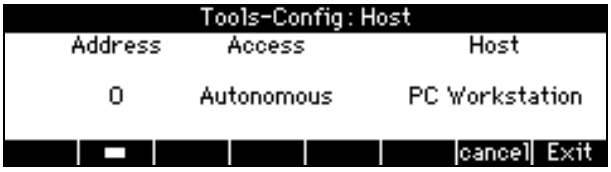


Figure 3-61 Setting Host Parameters

The mode of access is specified on the workstation. It cannot be changed on the instrument. The modes are:

- **Autonomous**—Instrument is not connected to the workstation software (stand alone operation)
- **Remote**—Instrument is controlled primarily from the workstation. You must start the instrument from the instrument keypad. You must prime the system, run diagnostics and select the type of chemistry from the instrument keypad.
- **Local**—Instrument is controlled from the instrument keypad or the workstation. You can edit sequences and control the instrument from either the instrument or the workstation. You may start the instrument from either the instrument keypad or the workstation. As with Remote mode, you must prime the system, run diagnostics and select the type of chemistry from the instrument keypad.

The host is also specified on the workstation. It cannot be changed on the instrument. The host may be a PC workstation.

Modes of Operation

The capabilities of the modes of operation of the Expedite instrument and the workstation are described in Table 3-34.

Table 3-34 Modes of Operation

Feature	Location	Mode		
		Autonomous	Remote	Local
Edit sequence	Instrument	Yes	No	Yes
	Workstation	N/A	Yes	Yes
Start instrument	Instrument	Yes	Yes	Yes
	Workstation	N/A	No	Yes
Stop instrument	Instrument	Yes	Yes	Yes
	Workstation	N/A	Yes	Yes
Run sequence	Instrument	Yes	No	Yes
	Workstation	N/A	Yes	Yes
Run diagnostics	Instrument	Yes	Yes	Yes
	Workstation	N/A	No	No
Prime	Instrument	Yes	Yes	Yes
	Workstation	N/A	No	No
Monitor status	Instrument	Yes	Yes	Yes
	Workstation	N/A	Yes	Yes
Set configuration	Instrument	Yes	No	Yes
	Workstation	N/A	Yes	Yes

System Information

Use the Ver option to display information about the current version of the system. When you select Ver, the screen shown in Figure 3-62 is displayed.

Figure 3-62 System Information Display

The following information is listed:

- Firmware version, date, and time
- Software version, date, and time

The only function assigned to the soft keys is Exit.

Naming the Instrument

The Name option allows you to specify a name for the instrument.

NOTE: *User-generated instrument names are displayed in upper and lower case and may contain up to eleven characters.*

When you select Name in the Tools Configuration menu, the screen shown in Figure 3-63 is displayed.

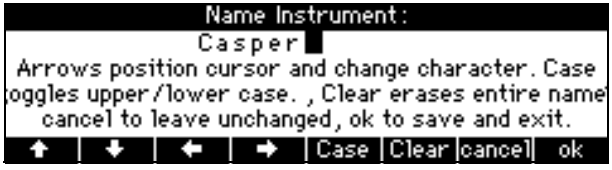


Figure 3-63 Naming the Instrument

The functions that are assigned to the soft keys to enable you to name the instrument are described in Table 3-11.

Table 3-35 Instrument Naming Keys

Key #	Label	Function
1	↑	Increments (A to Z followed by 0 to 9 and space) the value of the character at the cursor.
2	↓	Decrements (9 to 0 followed by Z to A) the value of the character at the cursor.
3	←	Moves the cursor one position to the left.
4	→	Moves the cursor one position to the right.
5	Case	Toggles the current character between upper and lower case.
6	Clear	Erases the entire instrument name.
7	Cancel	Returns you to the previous screen without saving the new name.
8	OK	Saves the new name and returns you to the previous screen.

Instrument Configuration Report

The Instrument Configuration lists various instrument parameters. Select **Print** on the Tools Configuration menu to print out the environment report. The following information is included in the report:

- Instrument status
- System version information
- Current system resources
- Protocols on the instrument
- Current user profile
- Sequences associated with current user profile

3.6.6 Specifying a User Profile

The instrument software allows you to set up eight user profiles that contain user preferences and sequences. Use the User Profile facility to specify default running parameters and protocols that will be in effect for every synthesis that is run when that Profile is selected.

When you select Profile, the screen shown in Figure 3-64 is displayed.

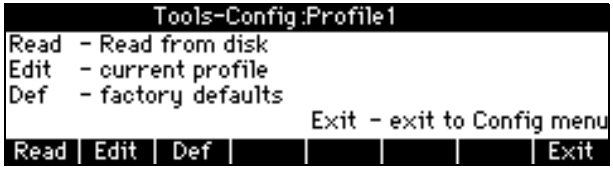


Figure 3-64 User Profile Options

The functions that are assigned to the soft keys in this screen are described in Table 3-36.

Table 3-36 User Profile Menu

Key #	Name	Function
1	Read	To select a user profile from the disk.
2	Edit	To modify the currently selected profile.
3	Def	To reset the parameters in the current profile to the factory defaults.
8	Exit	To return to the Configuration menu.

Selecting a User Profile

When you select Read from the User Profile menu, the screen shown in Figure 3-65 is displayed.

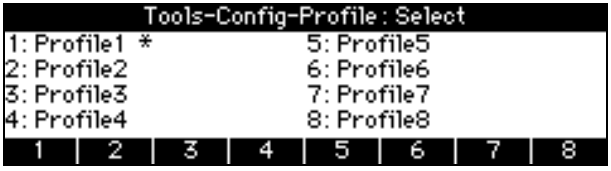


Figure 3-65 Selecting a User Profile

NOTE: *The asterisk (*) beside Profile 1 indicates that it is the profile currently in use.*

Press the appropriate key to select the desired profile.

Editing the User Profile

Use the Edit option to change the parameters in the currently selected User Profile. When you select Edit, the screen shown in Figure 3-66 is displayed. The functions that are assigned to the soft keys in profile editing are described in Table 3-37.

Table 3-37 Editing the User Profile

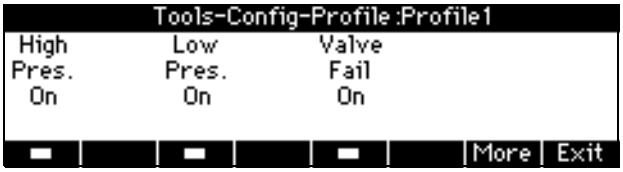
Key #	Name	Function
First Screen Keys		 ***Figure 3-66 User Profile—First Screen***
1	High Pres.	To enable or disable the High Pressure alarm (see Section 4.2, Gas Leak Diagnostics). The default is enabled (On).
3	Low Pres.	To enable or disable the Low Pressure alarm (see Section 4.2, Gas Leak Diagnostics). The default is enabled (On).
5	Valve Fail	To enable or disable the Valve Failure alarm. The default is enabled.
7	More	To provide access to additional options in the User Profile (see Figure 3-67).
8	Exit (All Screens)	To leave the user profile facility. When you select Exit you are given the following options: • **no**—Exit without saving the changes to the user profile • **cancel**—Continue editing the user profile • **ok**—Save the new profile and return to the Configuration menu

Table 3-37 Editing the User Profile (Continued)

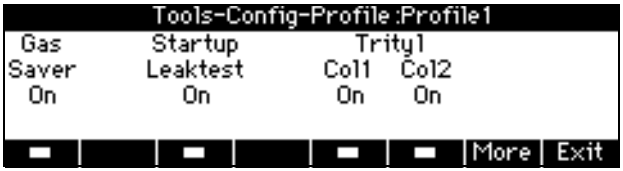
Key #	Name	Function
Second Screen Keys		 ***Figure 3-67 User Profile—Second Screen***
1	Gas Saver	To enable or disable the automatic gas saver. The gas saver performs a leak test when the instrument is turned on and periodically repeats the test during operation (see "Gas Saver" on page 3-70). The default is enabled.
3	Startup Leaktest	To enable or disable the automatic leak test when a synthesis is started (see "Startup Leak Test" on page 4-10). The default is enabled.
5	Trityl Col1	To enable or disable the Trityl Fail Sensor on column 1. The default is enabled.
6	Trityl Col2	To enable or disable the Trityl Fail Sensor on column 2. The default is enabled.
7	More	To provide access to additional options in the User Profile (see Figure 3-68).

Table 3-37 Editing the User Profile (Continued)

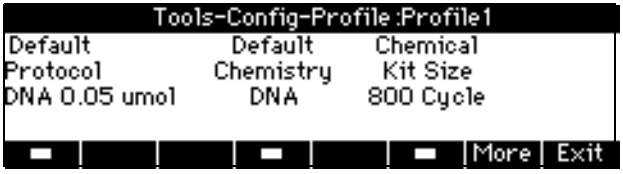
Key #	Name	Function
Third Screen Keys		 ***Figure 3-68 User Profile Third Screen***
1	Default Protocol	To select the default protocol. The available options are based on the selected chemistry. The following protocols are available for DNA chemistry: • 0.05 μmol (default) • 0.2 μmol • 1 μmol • 15 μmol • Use last—the last protocol that was run on the instrument ***NOTE:*** *The Protocol can still be changed at run-time.*
4	Default Chemistry	To select the default chemistry. The following are currently available: • DNA (Default) • RNA (DNA/RNA hybrids) • THIOATE—Phosphorothioated DNA • USER—User defined

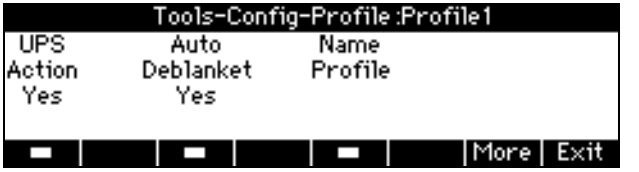
Table 3-37 Editing the User Profile (Continued)

Key #	Name	Function
6	Chemical Kit Size	To specify the size of the chemical kit. The following are available: • 150 Cycle—Model 8905 • 400 Cycle—Model 8909 • 800 Cycle—Model 8909 ***NOTE:*** *The 400 cycle kit allows you to use 0.5 gram quantities of monomers. The ancillary reagent volumes are the same as the 800 cycle kit (see Table 3-29).*
7	More	To provide access to additional options in the User Profile (see Figure 3-69).
Fourth Screen Keys		 ***Figure 3-69 User Profile—Fourth Screen***
1	Remove DMT	To specify the default disposition of the 5' DMT. The options are: • Remove the final DMT group from the oligomer • Leave the final DMT group on the oligomer (default) ***NOTE:*** *This can also be changed when you start a synthesis.*
3	Auto Restart	To specify if the synthesis is to be automatically restarted after a power failure. The default is no.
5	Reverse Video	Toggles the display between reverse video and normal display. The default is normal display.

Table 3-37 Editing the User Profile (Continued)

Key #	Name	Function
7	More	To provide access to additional options in the User Profile (see Figure 3-70).
Fifth Screen Keys		 ***Figure 3-70 User Profile—Fifth Screen***
1	Sequence Grouping	To specify the number of nucleotides that are grouped together in the sequence editor. Each time you press this key the number of nucleotides to be grouped together is increased by one. The range is 0 to 10. The default value is 3.
3	Sequence Display	To specify the way the sequence is displayed in the sequence editor. The display toggles between: • 3'-5' direction • 5'-3' direction (Default)
5	Universal Support	Select No to specify that the support has the first base attached. Select Yes to specify that the 3' base is coupled to the support on the instrument. The default is No.
7	More	To provide access to additional options in the User Profile (see Figure 3-71).

Table 3-37 Editing the User Profile (Continued)

Key #	Name	Function
Sixth Screen Keys		 ***Figure 3-71 User Profile—Fifth Screen***
1	UPS Action	To set parameters for the uninterruptible power supply option. Select UPS Action to specify the action to be taken if there is a power failure. The following options are available: • **Yes**—stop the synthesis at the end of the cycle (Default) • **No**—continue with the synthesis
3	Auto Deblanket	To enable or disable the automatic shut off of the blanket gas when the Bottle Change Tool for the A-train reagents is displayed. The default is enabled.
	Name Profile	Allows you to specify a unique name for the current profile. The procedure for specifying a profile name is the same as that for specifying an instrument (see "Naming the Instrument" on page 3-85) or sequence name. User generated instrument names are displayed in upper and lower case and may contain up to eleven characters.
7	More	To provide access to additional options in the User Profile (see Figure 3-66).

Naming the User Profile

Use the Name Profile option (located on sixth profile screen) to assign a name to a User Profile.

The procedure for specifying a profile name is the same as that for specifying an instrument (see "Naming the Instrument" on page 3-85) or sequence name. User generated instrument names are displayed in upper and lower case and may contain up to eleven characters.

Resetting to the Factory Defaults

Use the Default option to reset the parameters in the current User Profile to the factory default values specified. When you select this option, the screen shown in Figure 3-72 is displayed and you are requested to confirm your selection.

Figure 3-72 Confirming the Reset to Factory Defaults

At this time you may:

- Press **ok** to save the profile with the factory defaults.
- Press **cancel** to exit without saving the changes to the user profile.

3.6.7 Changing the Chemistry

The default chemistry on the Expedite Nucleic Acid Synthesis System is the β-cyanoethyl DNA Phosphoramidite method. This chemistry is specified in the default User Profile. If you wish to perform a synthesis using either the Thioate or RNA synthesis methods, perform the following steps to select and modify the user profile:

1. In the Main menu, select **Tools**.
2. In the Tools menu, select **Config**.

3. In the Configuration menu, select **Profil**.
4. In the Profile menu select **Edit**.

   The first User Profile screen is displayed.
5. Select **More**.

   The screen shown in Figure 3-73 is displayed.

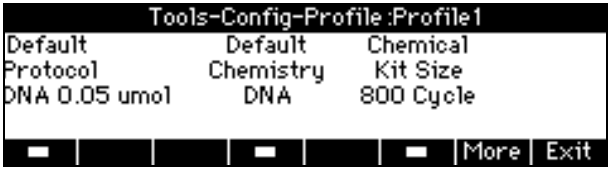
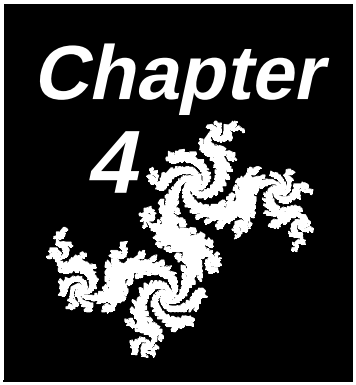


Figure 3-73 Chemistry Selection Screen

6. Press the soft key under the **Default Chemistry** option to cycle through the available options which are:

   - DNA
   - RNA
   - THIOATE
   - USER
7. Select the desired chemistry then press **Exit** followed by **ok** to save the new profile.
8. Press **Exit** repeatedly until the Main menu is displayed.

   The selected profile is the current profile. The parameters specified in this profile will be in effect for every synthesis that is run until a new profile is selected.



4 Maintenance and Troubleshooting

This chapter contains the following sections:

4.1 Routine Maintenance

Proper attention to maintenance will result in increased instrument life and performance. Regular maintenance will help the Expedite Nucleic Acid Synthesis System to continue to run in top condition.

The procedures listed in Table 4-1 should be performed when indicated.

Table 4-1 Routine Maintenance

Event	Procedure
Change of Chemistry	1. Install bottles containing acetonitrile in each reservoir position. 2. Run the **Prime All** routine **three** times (see Section 3.5.2, Prime All). 3. Change the end-line filters. 4. Install the new reagents. 5. Run the **Prime All** routine 3 times on each column.
Short Term Shutdown (less than 2 weeks)	Run the **Shutdown** prime routine (see Section 3.5.7, Shutdown) to rinse the system with solvent and blow the lines dry. See Table 3-20, "Prime Menu Routines," on page 3-46, for a description of the shutdown routine.
Long Term Shutdown (more than 2 weeks)	Run the **Shutdown** prime routine (see Section 3.5.7, Shutdown) to backflush the instrument with acetonitrile and blow the common passages dry.
Gas Pressure <500 psi	Change the helium, argon, or nitrogen tank.

4.1.1 Filter and O-ring Maintenance

Filters and O-rings must be inspected regularly and replaced when necessary.

End-line Filters

Polyethylene filters are located in the reagent reservoirs at the end of each fluid line. Inspect these end line filters regularly and replace them every six months or when system performance is diminished (product yield is low, trityls drop off quickly).

***NOTE**: If you suspect that your amidites contain moisture, replace the filters before you install new amidites on the system.*

O-rings

O-rings are located in the caps of the ancillary reagent and amidite reservoirs. Inspect these O-rings regularly and replace them every six months or when necessary.

To insert an O-ring:

1. Remove the old O-ring by prying it from the groove in the cap.

   ***Hint**: Use a dull probe or a paper clip to remove the O-ring.*

2. Discard the old O-ring.
3. Slip the new O-ring over the dip tube.
4. Insert the new O-ring completely into the groove of the cap. This can be facilitated by coating the O-ring with a very thin coating of silicon grease.

4.1.2 Checking the Fuses

The fuses are located in the power entry module on the lower right portion of the rear panel (see Figure 4-1).

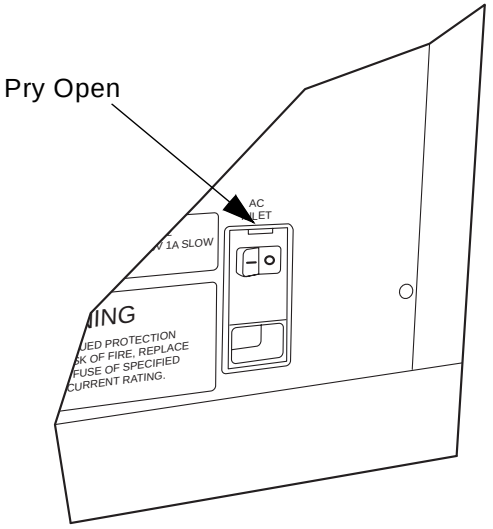


Figure 4-1 Location of the Power Entry Module

To remove the fuse carrier:

1. Remove the power cord from the instrument.
2. Pry the power entry module cover open and slide the two fuse carriers out of the instrument (see Figure 4-2).

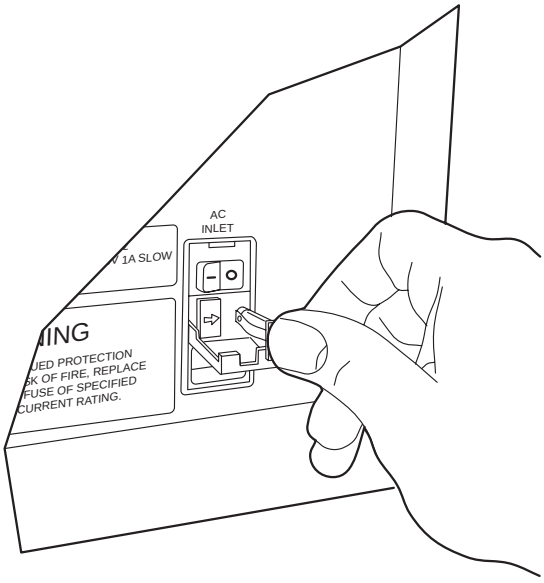


Figure 4-2 Checking the Fuses

3. Check that the proper fuses are installed. The two fuses are 2A (5 x 20 mm).

NOTE: *No voltage selection is required. The Expedite Nucleic Acid Synthesis System is equipped with a universal power supply that can be used for 100–240 VAC, 47–63 HZ operation. The use of a surge suppressor between the instrument and the electrical outlet is recommended.*

4.1.3 Waste Disposal

WARNING

Most of the reagents and solvents used in nucleic acid synthesis are hazardous. Wear a lab coat, gloves and eye protection when handling reagents.

The waste generated by nucleic acid synthesis is very flammable. Adequate ventilation is essential. Working under a fume hood is recommended.

AVERTISSEMENT

La plupart des réactifs et des solvants employés en synthèse d'acides nucléiques sont dangereux. Portez une blouse, des gants et des lunettes de protection lorsque vous les manipulez.

Les rejets produits par la synthèse d'acides nucléiques sont extrêmement inflammables. Une ventilation adéquate est indispensable. Il est recommandé de travailler sous une hotte.

Nucleic acid synthesis waste contains chlorinated and very flammable organic solvents and acids.

Les rejets provenant de la synthèse d'acides nucléiques contiennent des acides et des solvants organiques chlorés qui sont trés inflammables.

The Expedite system includes two waste bottles:

- 1 L bottle (TRT1 and TRT2 waste lines)—Chlorinated waste
- 4 L bottle (ORG1 and ORG2 waste lines)—Organic waste

Keep waste bottles in the spill tray provided with the system. Dispose of all waste frequently and in accordance with federal, state and local regulations.

Organic Waste

The non-chlorinated waste (ACN, THF, and dissolved amidites) is contained in the 4 L waste bottle. Waste lines are labeled ORG1 and ORG2. Keep the flammable organic waste away from flame or sparks. Empty the organic waste after each synthesis to eliminate the danger of a reservoir overflow.

Les rejets non chlorés (ACN, THF et amidites en solution), qui sont extrêmement inflammables sont contenus dans un flacon de 4 l. Ces rejets doivent être tenus à l'écart de toute flamme ou étincelle. Videz ce récipient aprés chaque synthèse pour éviter le risque de voir le réservoir déborder.

Chlorinated Waste

The chlorinated waste (DCM, TCA, hazardous organic solvents and organic acids) is contained in the 1 L waste bottle. Waste lines are labeled TRT1 and TRT2. Chlorinated waste can burn skin and eyes when concentrated. Dispose of chlorinated waste in accordance with federal, state and local regulations.

Les rejets chlorés contiennent des solvants organiques dangereux et des acides (DCM et TCA) susceptibles de provoquer des brûlures de la peau et des yeux quand ils sont concentrés. Les déchets chlorés doivent être détruits conformément aux directives en vigueur dans ce domaine.

4.1.4 Gas Cylinder Replacement

Use a dry helium gas supply for best performance. Dry helium is less soluble in the reagents and ensures a longer life for the chemicals, especially the monomers.

***NOTE**: If helium is not available, you can use argon or nitrogen (high purity, 99.995%). However, bubbles introduced by these gases may cause trityl failures.*

To replace the cylinder:

1. Decrease the gas pressure on the 2-stage cylinder regulator to zero (0).
2. Turn the gas off at the cylinder.
3. Disconnect the gas supply line from the rear of the instrument cabinet by pressing the quick release tab (see Figure 4-3).

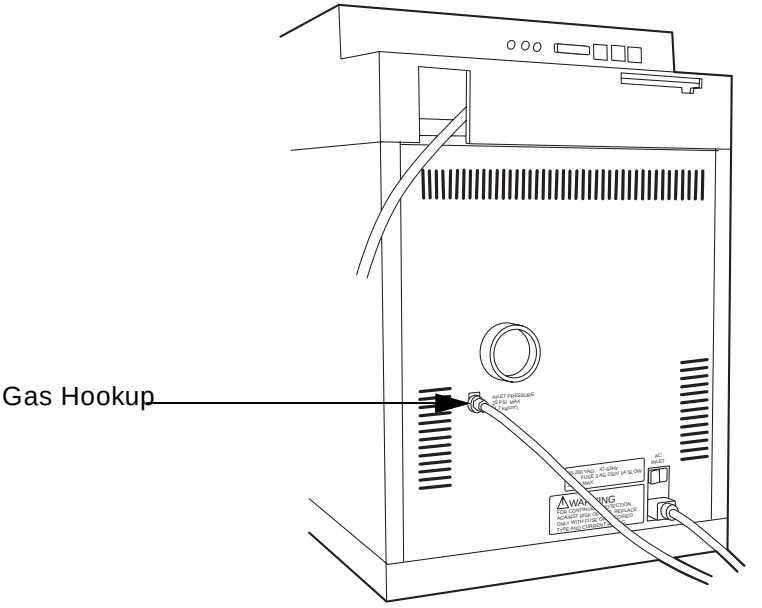


Figure 4-3 Rear of Instrument Cabinet

4. Remove the regulator from the cylinder.
5. Install the regulator on the new cylinder.
6. Reconnect the gas line to the reagent instrument cabinet and increase the gas pressure slowly to 20 psi.

CAUTION

Increasing the pressure too quickly may damage the internal gas pressure system.

4.2 Gas Leak Diagnostics

The Expedite system has high and low gas pressure sensors. These sensors are activated by default. However, you may turn them off by editing the User Profile (see Section 3.6.6, Specifying a User Profile).

High Pressure Sensor

The high pressure sensor continuously monitors the input gas pressure. If the gas pressure sensor is activated and the pressure falls below a specified level (10 psi), the “High pressure system failure” message (see Figure 4-4) appears.

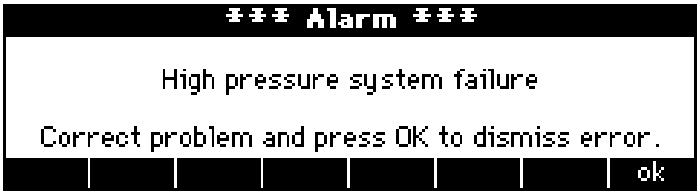


Figure 4-4 Gas Supply Failure

You must respond to the warning before further operation is allowed. If you fail to acknowledge the warning by pressing the **ok** key, the synthesis is halted at the first “safe point.” The failure is also recorded in the Instrument History Log.

Low Pressure Sensor

The low pressure sensor measures the severity of gas leaks when the instrument is active or idle. These leaks are typically caused by removing bottles or attaching bottles to caps with a defective O-ring. If the gas pressure sensor has been activated in the User Profile (see Section 3.6.6, Specifying a User Profile), the low pressure switch is activated when the pressure drops below 4.0 psi. If the low pressure switch is activated during a synthesis, the “Low pressure system failure” message is displayed. If the low pressure switch is activated when the instrument is idle, the “Gas saver low pressure leak failure” message shown in Figure 4-5 is displayed.

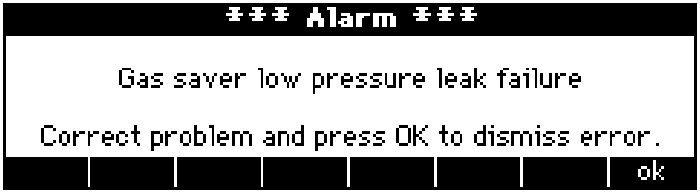


Figure 4-5 Low Pressure Failure—Instrument Idle

You must respond to the warning before further operation is allowed. If you fail to acknowledge the warning by pressing the **ok** key, the synthesis is halted at the first "safe point." The failure is also recorded in the Instrument History Log.

Gas Leak Detection

The Diagnostics menu contains tools for the diagnosis and isolation of leaks. The Diagnostics tools include routines for independent leak testing of the High and Low pressure systems (see Section 3.6.1, Diagnostic Routines).

Startup Leak Test

The Startup Leak Test automatically runs a shortened version of the gas leak diagnostic test when you press *Start* to begin a synthesis. If the test fails, a warning is issued and the instrument is halted at the end of the next cycle. The Startup Leak Test may also be enabled or disabled by editing the User Profile (see Section 3.6.6, Specifying a User Profile).

Gas Leak Isolation

Gas leak warnings must be acknowledged and the leak located and repaired before you continue with a synthesis. Table 4-2 contains some suggestions that you should try before contacting PerSeptive Biosystems Technical Support or your PerSeptive Biosystems Service Representative.

Table 4-2 Gas Leak Isolation Tests

Alarm	Possible Cause	Solution
High Pressure Failure High Pressure and Low Pressure Failure	Gas tank empty	Check tank regulator gauge. Replace the tank if necessary.
	Large leak at the inlet connections	1. Check the tank regulator connections. 2. Check the quick connect at the rear of the instrument.
	Defective high pressure isolation valve	Call your PerSeptive Biosystems Service Representative.
Low Pressure Failure	Missing reagent bottle or loose cap	Check all the bottles to make sure the connections are finger tight. Do not overtighten the bottles because this deforms the O-rings and may cause gas leakage.
	Missing O-ring	Place the appropriately sized O-ring in the bottle cap assembly.
	Low pressure gauge pressure regulator adjusted incorrectly	Call your PerSeptive Biosystems Service Representative.
	Defective low pressure isolation valve	Call your PerSeptive Biosystems Service Representative.
	Defective connection between pneumatics and fluidics	Call your PerSeptive Biosystems Service Representative.
	Leak in internal pneumatic tubing	Call your PerSeptive Biosystems Service Representative.

4.3 Flow and Volume Test

Overview

The Flow and Volume test checks the fluidic pathways:

- **Volume test**—Pulses the selected reagent injector approximately 125 times and measures the volume delivered.
- **Flow test**—Opens inlet and outlet solenoids for approximately 10 seconds and allows blanket pressure (6 psi) to force fluid through the selected column fluidic path.

When to perform

Perform the Flow and Volume test if you are having performance problems such as trityl failures, low trityl intensity, or poor oligonucleotide quality.

The Flow and Volume test can help you determine if decreased performance is due to mechanical problems.

Equipment required

To perform the Flow and Volume test, you need:

- 10 ml graduated cylinder
- Column unions
- Gloves, safety glasses, and lab coat
- 500 ml actetonitrile

***NOTE**: If you do not have acetonitrile available, you can perform the Flow and Volume test with synthesis reagents. However, approximately 20 ml of each reagent is consumed during the test.*

Preparing the system

To prepare the system for the Flow and Volume test:

1. Remove the synthesis reagents from the system and replace with approximately 20 ml of acetonitrile in each reservoir.
2. Replace columns with unions.
3. Unscrew the four waste lines from the waste caps and place Pall lines in the 10 ml graduated cylinder.

Results worksheet

Make a copy of the worksheet below and use it to record the results of the Flow and Volume test.

Table 4-3 Result Worksheet for Flow and Volume Test

NOTE: *Make a copy of this worksheet and record results on the copy.*				
Reservoir	**Volume Test (ml)**		**Flow Test (ml)**	
	Col1	**Col2**	**Col1**	**Col2**
Wash A				
Wash B				
Cap A				
Cap B				
Auxiliary				
Oxidizer				
Deblock				
Wash				
Activator				
Monomer 9				
Monomer 8				
Monomer 7				
Monomer 6				
Monomer 5				
Monomer T				
Monomer G				
Monomer C				
Monomer A				

Performing Flow and Volume test on Column 1

To perform the Flow and Volume test:

WARNING

Wear gloves, safety glasses, and lab coat when you perform the Flow and Volume test. DBLK (Deblock solution containing trichloroacetic acid [TCA]) is delivered to the waste lines during this test. TCA is highly caustic.

AVERTISSEMENT

Portez des gants, des lunettes de protection, et une blouse de laboratoire, quand vous effectuez le test de Flux et Volume. Le DBLK (Solution de déblocage contenant de l'acide trichloracétique [TCA]) est distribué aux lignes d'écoulement pendant ce test. Le TCA est très caustique.

1. Select **Tools** from the main menu, then select **Diag**.
2. Select **Fluid**.
3. Select Column **1**. The Diagnostic Mode Prime Individual screen is displayed (Figure 4-6).

Figure 4-6 Diagnostic Mode Prime Individual Screen

4. Select the reagent to test. Press More if the reagent you are testing is not displayed. The Diag Mode Prime Status screen is displayed (Figure 4-7).

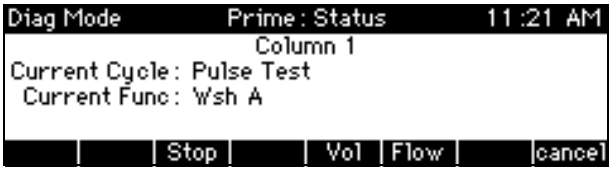


Figure 4-7 Diag Mode Prime Individual Screen

5. Press **Volume**. The Volume test runs.
6. Record the volume of reagent collected. Empty the graduated cylinder.
7. Repeat the Volume test and record the volume of reagent collected. If the volume is not within ±0.1 ml of the first measurement, repeat the Volume test a third time.
8. Press **Flow**. The Flow test runs.
9. Record the volume of reagent collected. Empty the graduated cylinder.
10. Repeat the Flow test and record the volume of reagent collected. If the volume is not within ±0.1 ml, repeat the Flow test a third time.
11. Repeat step 4 through step 10 for all reagents.
12. Compare the volumes delivered to the volumes listed in "Results" on page 4-16.

Verifying Wash Volume on Column 2

It is not necessary to perform the Flow and Volume test on all reagent positions for column 2. Acceptable results on column 1 indicate that the system reagent injectors and solenoids are functioning properly.

However, it is necessary to verify that the system is delivering the same volumes for column 1 and column 2. Do this by:

- Performing the Flow and Volume test 2 times on the Wsh reagent position for column 2 (perform a third time if volumes are not within ±0.1 ml)
- Comparing the volume delivered to the volume delivered for column 1

If the volume delivered for column 2 is not within ±0.1 ml of the volume delivered for column 1, it may indicate a blockage in the fluidic system. Call PerSeptive Biosystems Technical Support.

Results

Table 4-4 lists the acceptable ranges for Flow and Volume tests. If your results are not within the ranges listed, call PerSeptive Biosystems Technical Support.

Table 4-4 Acceptable Ranges for Flow and Volume Test

Test	Reagent	Acceptable Range (ml)	Comments
VOL	All except Cap A and Cap B	1.6–2.4	_____
	Cap A and Cap B	3.1–4.7	Volume delivered is double the volume for other reagents because Cap A and Cap B reagents are delivered simultaneously.
FLOW	All	1.0–2.0	Volume of reagent delivered may vary slightly from instrument-to-instrument because flow is affected by blanket pressure setting.

4.4 Troubleshooting

Diagnosis and isolation of a problem is initially based on the concept of a single point failure. Troubleshooting involves:

- Identifying problems with the mechanical/electronic components of the system.
- Determining the cause of inconsistencies in synthesis quality (chemical problems).

After the problem has been identified, the possible causes can be reviewed, and corrective action taken.

Some symptoms, possible causes, and solutions to help you troubleshoot are listed in this section. However in the event that you cannot identify the problem yourself, call your PerSeptive Biosystems Service Representative or Technical Support Department.

4.4.1 Mechanical/Electronic Troubleshooting

This section lists some possible symptoms and methods for identifying problems with the mechanical/electronic components of the Expedite Nucleic Acid Synthesis System.

NOTE: *With the exception of the fuses, the Expedite Nucleic Acid Synthesis System has no user-serviceable electronic components. The following information is provided to help determine the cause of the problem.*

If you have a problem with your system, perform the troubleshooting procedures listed in Table 4-5 before calling your PerSeptive Biosystems Service Representative. If you call PerSeptive Biosystems for assistance, please have a description of the problem and a list of the procedures that you tried prior to placing the call.

Table 4-5 Mechanical and Electronic Troubleshooting Symptoms and Solutions

Symptom	Possible Cause	Solution
No power to system	Not connected to house voltage supply.	Plug in main power cord.
	Blown fuse.	Replace the fuses. See Section 4.1.2, Checking the Fuses.
Blank Display	Internal electronic fault due to power supply irregularities.	Contact your PerSeptive Biosystems Service Representative.
Anomalous software behavior	Faulty diskette or disk drive.	Insert the spare boot diskette provided in the startup kit. If the new diskette does not correct the problem, contact your PerSeptive Biosystems Service Representative.
Orange fluid in column drip tray and instrument cabinet drip tray	Leak in the trityl monitor.	Contact your PerSeptive Biosystems Service Representative.
Fluid in column drip tray and instrument cabinet drip tray	Column inserted incorrectly.	Check the column connections.

Table 4-5 Mechanical and Electronic Troubleshooting Symptoms and Solutions

Symptom	Possible Cause	Solution
Fluid in the instrument cabinet drip tray	Cracked reagent bottle.	Check all the reagent bottles for cracks. ***NOTE**: Wear gloves when handling the reagent bottles.*
	Leak in the fluidics plate.	Contact your PerSeptive Biosystems Service Representative.
"High pressure system failure" message on the screen	Depleted gas supply.	Replace gas tank. See Section 4.1.4, Gas Cylinder Replacement.
"Low pressure failure" message on the screen	Incomplete pressurization due to leaks at reservoirs or internal fittings.	See Table 4-2 Gas Leak Isolation Tests.
Improper delivery of reagents	Reduced or no flow due to clogged endline filters or crimped lines.	Change filters and check lines in reagent bottles and waste bottles.
	Reduced or no flow due to pressurization system fault.	See Table 4-2 Gas Leak Isolation Tests.
	Reduced or no flow due to valve failure or fluid line blockage.	Contact your PerSeptive Biosystems Service Representative.

4.4.2 Chemical Troubleshooting

Inconsistencies in synthesis quality can result from a number of chemical or mechanical problems. In some situations, it may be difficult to determine whether the cause is actually chemical or mechanical in nature. The first step in diagnosing a problem is to define the symptom.

Reagent Quality

Chemical problems are usually associated with outdated reagents or reagents that have become water contaminated due to improper storage. The nucleotide monomers, activator, and acetonitrile are the reagents that most strongly affect stepwise coupling efficiencies.

Reagent Delivery

The most common mechanical problem is improper delivery of reagents due to leaks or blocks in the system.

Coupling Efficiency

Low coupling efficiencies can be quantitatively determined through spectrophotometric analysis of the trityl fractions, and qualitatively determined through simple visual inspection of collected trityl fractions. In a successful synthesis, the second trityl has the strongest orange color. Subsequent trityls have a consistently decreasing color. A problem with the synthesis, and the point in the synthesis at which it occurred, is frequently indicated by a sharp drop in trityl color.

In the presence of water, bases, or alcohols the DMT groups will not be in the cationic state. Therefore, weakly colored trityl fractions may be the result of contaminated collection tubes. If this is the case, addition of 1 mL of Deblock Solution should bring back a strong color.

Product Analysis

Another symptom of a problematic synthesis, low overall product yield, can be determined by measuring the total Optical Density, at 260 nm, of an aqueous solution of the oligonucleotide after cleavage and deprotection. Other synthesis problems can be detected through interpretation of purification data (for example, unusual band patterns in gels or extra peaks in chromatograms).

After taking corrective action, perform a trial 5'-ACGTT-3' synthesis using a fresh “T” column to confirm that the problem has been rectified.

Table 4-6 lists some possible symptoms and causes of inconsistent synthesis quality and the appropriate remedy.

Table 4-6 Chemical Troubleshooting

Symptom	Possible Cause	Solution
Full Sequence not synthesized (too few trityl fractions, short oligomer on gels, HPLC)	Reagents depleted.	Refill empty bottles.
	Empty gas tank.	Check tank regulator gauge. Replace the tank if necessary.
	Clogged reagent or waste line or filter.	Check waste and reagent lines for a block. Replace reagent line filter.
Low coupling efficiencies due to mechanical problems (other symptoms include erratic or low volumes in trityl fractions, low overall yield, unusual band or peak patterns)	Low flow rates or no flow rates due to low pressure blanket system fault.	Perform a Flow and Volume test, described in Section 4.3, Flow and Volume Test. Call PerSeptive Biosystems Technical Support if the test fails.
	Low flow rates or no flow rate due to fluidic failure or fluid line blockage.	Perform a Flow and Volume test, described in Section 4.3, Flow and Volume Test. Call PerSeptive Biosystems Technical Support if the test fails.

Table 4-6 Chemical Troubleshooting (Continued)

Symptom	Possible Cause	Solution
Low coupling efficiencies due to chemical problems (other symptoms include low overall yield, unusual band or peak patterns)	Nucleotide monomers outdated or solution too old.	Replace any monomer which has been on the instrument for more than two weeks.
	Recently added reagent is at fault.	Replace the most recently added reagent.
	Water contaminated reagent (from exposure to atmosphere during loading, reagent transfer, or improper storage).	Perform trial 5'-ACGTT-3' syntheses, replacing the activator (tetrazole), wash (acetonitrile) and monomers one by one until the contaminated reagent is detected.
	Water contaminated reagent (from a slow leak in the pressurization system).	Run the gas leak diagnostics routine to confirm the presence of a gas leak and contact your PerSeptive Biosystems Service Representative.

4.5 Error Messages

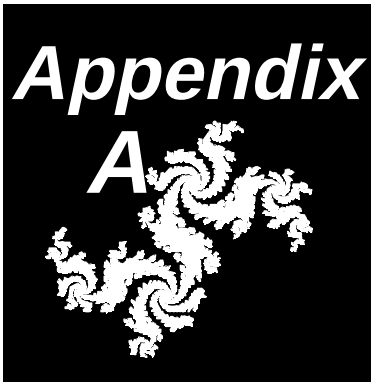
Table 4-7 describes the error messages that you may encounter when running the Expedite Nucleic Acid Synthesis System:

Table 4-7 Expedite System Error Messages and Corrective Action

Error Message	Corrective Action
“Internal error—#XX, ret_code—XX”	Call your PerSeptive Biosystems Service Representative.
“High pressure system failure”	See Table 4-2.
“Low pressure system failure”	See Table 4-2.
“Gas saver low pressure leak failure”	See Table 4-2.
“Startup low pressure leak failure”	See Table 4-2.
“Valve driver fault”	Indicates an electrical problem with the fluidic valve driver circuits. Run the LED test (see Section 3.6.1, Diagnostic Routines) then call your PerSeptive Biosystems Service Representative.
“Trityl total yield failure on column 1”	The trityl monitor senses that the coupling has failed. Check the trityl color. If the trityl monitor is functioning correctly, abort the synthesis.
“Trityl total yield failure on column 2”	
“Power Failure”	If a UPS is installed and the power fails, this message will appear in the log. The system will take the action specified in the user profile (see Section 3.6.6, Specifying a User Profile).

Table 4-7 Expedite System Error Messages and Corrective Action

Error Message	Corrective Action
“General disk failure”	Make sure that the boot diskette is inserted in the diskette drive. Reboot the instrument using the new system diskette provided in the startup kit.
“Printer not available”	Make sure that the printer is plugged in and turned on. Check the connection between the printer and the instrument.
“Bad sequence for protocol”	Sequence and chemistry or protocol are not compatible (for example, DNA sequence but RNA chemistry selected. Check the default chemistry (see Section 3.6.6, Specifying a User Profile) and the selected protocol (see Section 3.3.2, Running a Sequence).



A Installation

The 8900 Expedite Nucleic Acid Synthesis System must be installed by a qualified PerSeptive Biosystems representative. Contact your local PerSeptive Biosystems representative following delivery to make arrangements for installation of the unit.

Site Requirements

The instrument should be placed in a well lit room, out of direct sunlight. There should be an electrical outlet, space for a gas cylinder, and a fume hood or some suitable ventilation system close to the instrument.

The room temperature should be in the range of 20 to 30°C. Do not operate the instrument in a cold room. The protocols are optimized for room temperature operation and low temperatures may compromise synthesis results.

Bench Space and Support

Designed for bench-top use, the 8900 instrument cabinet requires 16 inches (40 cms) of linear bench space. The reagent tray for the Model 8909 requires 11.5 inches (29 cms). The space should be 20 inches (52 cms) deep with at least 24 inches (60 cms) of overhead clearance. The instrument cabinet weighs 102 pounds (46 kg).

Allow a clearance of 9 inches (25 cm) at the back for proper connection of power cords and gas lines, as well as proper ventilation. Allow additional space nearby for the waste tray and a printer (if used).

A

Gas Supply

You must provide a Helium tank (pre-purified grade 4.5) and a 2-stage regulator capable of providing 20 psi pressure. Helium is the recommended gas because it degases the reagent solutions so that bubbles are not generated during synthesis which can cause channeling of the fluid around the CPG supports.

If helium is unavailable, you may use dry Argon or dry Nitrogen.

Power Supply

The instrument contains a Universal Input power supply for voltages from 100–240 VAC 50/60 Hz. You are recommended to install a surge protected power strip between the instrument and the electrical outlet.

Unpacking Instructions

Examine the package for signs of damage before starting to unpack the instrument.

WARNING

The Expedite Cabinet weighs 102 pounds (46 kg). Two people are required to safely lift the instrument cabinet.

AVERTISSEMENT

L'appareil pèse 46 kg. Il est nécessaire d'être deux pour le soulever.

Unpack the instrument carefully and inspect the components and accessories for signs of damage. Inform the carrier if there are any indications that the instrument has not arrived in good condition.

Damage incurred during transit is the responsibility of the carrier, not the manufacturer, and must be reported to the carrier immediately. The original shipping containers must be available for inspection by the carrier if a claim for damage is to be filed. In any event, save the shipping containers, they provide excellent protection for the instrument in storage or in transit.

Shipping List

The shipping cartons should contain the following items.

- Expedite Nucleic Acid Synthesizer
- Startup Kit
  - User's Guide
  - Power cords
  - Fuses
  - Software disks (2)
  - Waste reservoir
  - Waste vent line assembly
  - Spare O-rings and filters
  - Column replacement fittings
  - Vent hose
  - Reagent reservoirs
  - RS422 Interconnection cable (for connection to the workstation)
  - Gas line assembly
- External reagent tray assembly (Model 8909)

Installation

Perform the following steps to install the instrument:

1. Place the instrument cabinet on the bench.
2. Make the electrical connections (see Figure A-1).
   - Make sure that the power cord has the correct plug for your voltage.
   - Attach the power cord to the rear of the instrument.
   - Plug the cord into the wall socket.
3. Hook up the gas.
   - Use the compression fitting provided to attach the inlet gas line to the tank pressure regulator (20 psi).
   - Attach the male quick disconnect fitting to the gas inlet on the rear of the instrument cabinet (see Figure A-1).

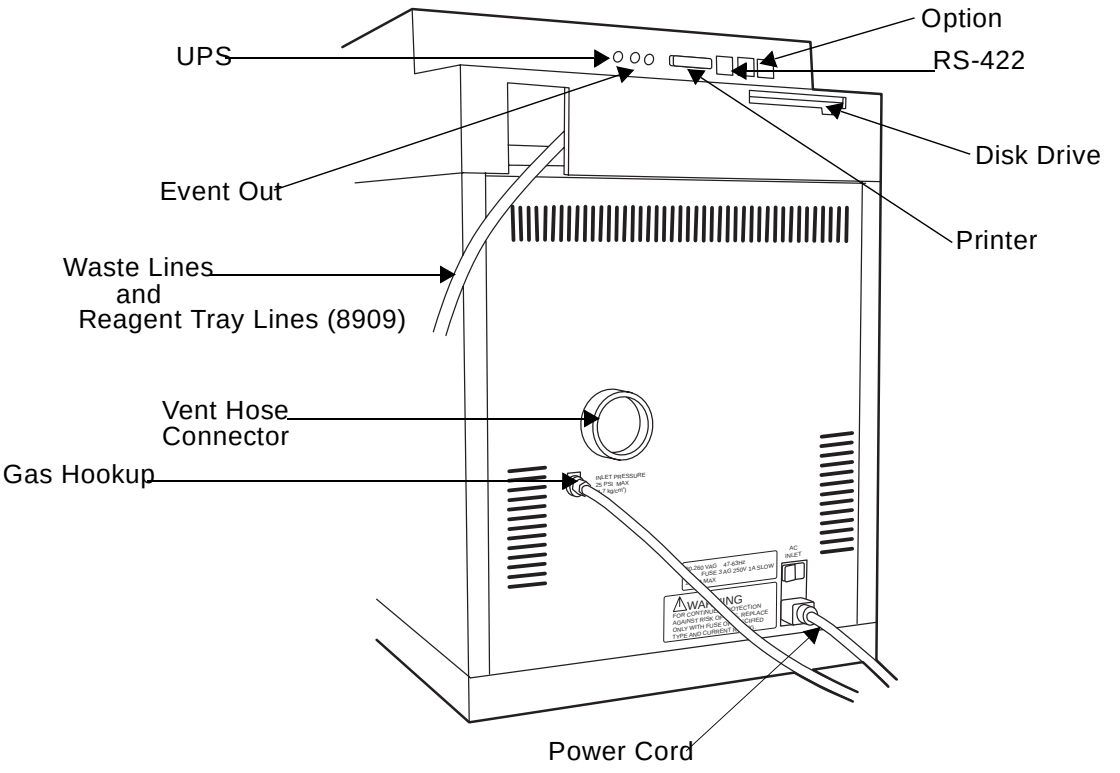


Figure A-1 Electrical Hookup and Gas Lines

4. Hook the vent hose to the connector on the rear of the system (Figure A-1). Connect the other end of the vent host to an exhaust hood.

5. Check the waste bottles. The waste lines exit the instrument cabinet at the right rear of the instrument (see Figure A-1).

   - Check the O-ring inside the waste bottle caps. Screw the ORG1 and ORG2 cap/waste lines onto the 4 L waste bottle. Screw the TRT1 and TRT2 cap/waste lines onto the 1 L waste bottle.
   - Make sure that the waste tubes are draining into the bottle.

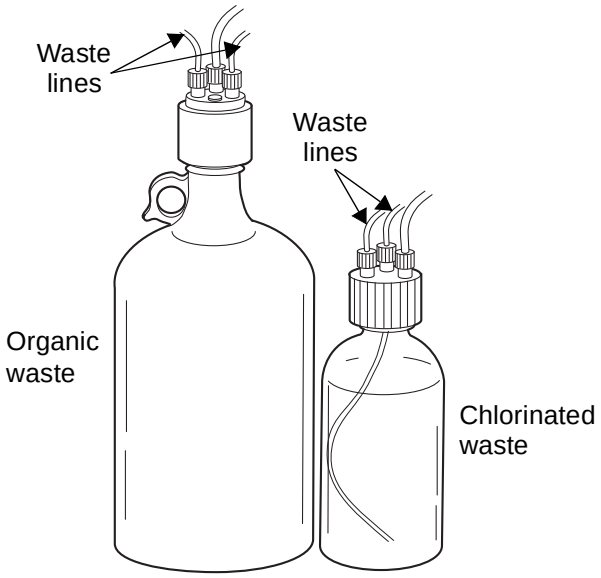


Figure A-2 Waste Bottle Cap Showing Waste and Vent Lines

6. Model 8909—connect the reagent tray to the instrument cabinet:

- Place the instrument tray on the bench beside the instrument cabinet.
- Place the Wash A and Deblock bottles in the caddy.
- Attach the caps on reagent lines, which exit the instrument cabinet at the right rear of the instrument (see Figure A-1), to the appropriate bottle:
  - Attach the line labeled “DB8” to the deblock bottle.
  - Attach the line labeled “WA7” to the Wash A bottle.

Installation

Pour installer votre appareil, procédez comme suit :

1. Placez l'appareil sur une paillasse.

2. Procédez au raccordement électrique (voir Figure A-1).
   - Assurez-vous que le fil d'alimentation possède la prise compatible avec la tension utilisée.
   - Raccordez le fil à l'arrière de l'appareil.
   - Branchez-le dans la prise.
3. Raccordement à la source de gaz
   - Utilisez l'écrou de compression fourni pour raccorder le tuyau d'alimentation en gaz au régulateur de pression de la bouteille 20 psi (1,7 bar).
   - Raccordez le raccord rapide mâle à l'entrée gaz à l'arrière de l'appareil (voir Figure A-1).
4. Flacons de rejet

   Les tuyaux de rejet sortent sur l'arrière droit de l'appareil (voir Figure A-1).

- Vérifiez les joints toriques à l'intérieur du bouchon du flacon de rejet, puis vissez complétement le bouchon sur le flacon (sur les deux flacons pour un appareil avec détecteur de trityl, voir Figure A-2).
- Assurez-vous que le tuyau de rejet se déverse bien dans le flacon.
- Raccordez le tube évent à une canalisation d'échappement (voir Figure A-1).

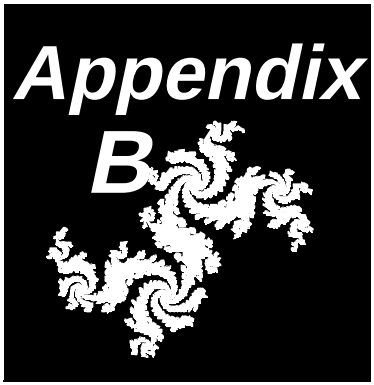
5. Modèle 8909 - Fixez le portoir à réactifs sur l'appareil :

- Placez le portoir sur la paillasse à côté de l'appareil
- Placez les flacons de solvant de lavage "Wash A" et de solution de détritylation "Deblock" dans le portoir.
- Raccordez les bouchons aux tuyaux de réactifs qui dépassent à l'arrière de l'appareil (voir Figure A-1) de la façon suivante :

  Le tuyau marqué "DB8" au flacon "Deblock" (solution de détritylation)

  Le tuyau marqué "WA7" au flacon "Wash A" (solvant de lavage).

A



B Reagent Safety

This appendix provides a summary of safety information for nucleic acid synthesis reagents. For complete information, refer to the MSDS (Material Safety Data Sheet) available from PerSeptive Biosystems.

WARNING

Most of the reagents and solvents used in nucleic acid synthesis are hazardous. Wear a lab coat, gloves and eye protection when handling reagents. Adequate ventilation is essential and working under a fume hood is recommended.

AVERTISSEMENT

La plupart des réactifs et solvants employés en synthèse d'acides nucléiques sont dangereux. Portez une blouse, des gants et des lunettes de protection lorsque vous manipulez des réactifs. Une ventilation adéquate est nécessaire et il est recommandé de travailler sous une hotte.

B

Acetonitrile

A colorless hygroscopic liquid, extremely flammable, harmful if swallowed, inhaled or absorbed through the skin. Vapor or mist is irritating to the eyes, mucous membranes and upper respiratory tract. Symptoms of exposure may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, nausea and vomiting.

First Aid: Remove contaminated clothing and shoes. Flush eyes or skin with plenty of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician. Wash contaminated clothing before reuse.

Recommended Storage: Store under nitrogen in a cool dry place.

Fire and Explosion Data: Flash point: 5°C. Extinguish with CO_2, dry chemical powder, alcohol, or polymer foam. Emits toxic fumes under fire conditions. Wear self-contained breathing apparatus and protective clothing. Use water to cool fire exposed containers. Vapors can flow along surfaces to a distant ignition source and flash back. Closed containers exposed to heat may explode.

Spill or Leak Procedures: Evacuate the area. Shut off all sources of ignition and ventilate the area. Cover with an activated carbon absorbent, scoop into a closed container and transport outside. Ventilate area and wash spill site after material pickup is complete.

Acetic Anhydride

Acetic anhydride is a colorless liquid with a strong odor. It is harmful if swallowed, inhaled, or absorbed through the skin. It is extremely destructive to the tissue of the mucous membranes and upper respiratory tract, eyes and skin. Inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema. Symptoms of exposure may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea and vomiting.

First Aid: Remove contaminated clothing and shoes. Flush eyes or skin with plenty of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician. Discard contaminated shoes. Wash contaminated clothing before reuse.

Recommended Storage: Keep tightly closed. Store in a cool dry place.

Fire and Explosion Data: Extinguish with CO_2, dry chemical powder, alcohol or polymer foam. Wear-self contained breathing apparatus, rubber boots and heavy rubber gloves to prevent contact with the skin and eyes.

Spill or Leak Procedures: Evacuate area. Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Cover with activated carbon adsorbent, take up and place in closed containers. Transport outdoors. Ventilate area and wash spill site after material pickup is complete.

Activator Solution - Amidite Chemistry

This solution contains *1H-tetrazole* and *acetonitrile.* See the individual reagents for details of safety precautions. This is a flammable, hygroscopic solution. Keep away from open flames and avoid breathing the vapors.

Recommended storage**:** Store at room temperature, away from light.

Ammonium Hydroxide

Harmful if swallowed, inhaled or absorbed through the skin. This strong base is extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes and skin. Inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema. Symptoms of exposure may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea and vomiting.

First Aid: Remove contaminated clothing and shoes. Flush eyes or skin with plenty of water for at least 15 minutes. Call a physician. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.

Recommended Storage: Use fresh ammonium hydroxide (open less than 2 months). To ensure a concentrated solution after opening, aliquot the ammonium hydroxide in small volumes, cap tightly, and store in a freezer.

Fire and Explosion Data: Noncombustible, emits toxic fumes under fire conditions.

Spill or Leak Procedures: Evacuate area. Wear self-contained breathing apparatus. Absorb on sand or vermiculite, take up and place in closed containers. Ventilate area and wash spill site after material pickup is complete.

t-Butylphenoxy Acetic Anhydride

t-Butylphenoxy acetic anhydride (tBPA) and tetrahydrofuran (THF) are components of the Expedite Cap A solution. THF is a flammable solvent which will form explosive peroxides over a period of time in the presence of oxygen. It may be harmful by inhalation, ingestion, or skin absorption. Vapor or mist is irritating to the eyes, mucous membranes and upper respiratory tract. Causes skin irritation. Exposure can cause coughing, chest pains, difficulty in breathing, nausea, dizziness and headache. The hazards from exposure to tBPA alone are not known. For further information on Cap A, see "Tetrahydrofuran (THF)" on page B-9.

Recommended Storage: Store Expedite Cap A solution at room temperature, away from light. Dispose of opened bottles that are over six months old.

Cap A (Amidite Capping Solution)

This solution contains *acetic anhydride* and *tetrahydrofuran (THF)*. See the individual reagents for details of safety precautions. THF is a flammable solvent and is irritating to eyes, skin, and mucous membranes. It will form explosive peroxides over a period of time in the presence of oxygen.

Recommended Storage: At room temperature, away from light. Dispose of opened bottles that are over six months old.

Cap A (Expedite Capping Solution)

This solution contains *t-butylphenoxy acetic anhydride* and *tetrahydrofuran (THF)*. See the individual reagents for details of safety precautions. THF is a flammable solvent and is irritating to eyes, skin, and mucous membranes. It will form explosive peroxides over a period of time in the presence of oxygen.

Recommended Storage: At room temperature, away from light. Dispose of opened bottles that are over six months old.

Cap B (Capping Activator)

This solution contains *1-N-methylimidazole, pyridine,* and *tetrahydrofuran (THF).* See the individual reagents for details of safety precautions.

Recommended Storage: At room temperature, away from light. Dispose of opened that are over six months old.

Deblock Solution

This solution contains *trichloroacetic acid (TCA)* and *dichloromethane (DCM).* See the individual reagents for details of safety precautions.

Recommended Storage: Keep tightly closed. Store in a cool dry place.

Dichloromethane (DCM) (Methylene Chloride)

A colorless clear liquid with mild chloroform-like odor. Harmful if inhaled, irritating to skin, eyes, and mucous membranes. May cause nausea, vomiting, light-headedness, or headache. Inhalation of vapors in high concentration causes narcosis.

First Aid: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. If ingested and if conscious, induce vomiting with water. Keep airway clear. Call a physician. Flush eyes with plenty of water for at least 15 minutes. Wash skin with soap and water. Wash contaminated clothing before reuse.

Recommended Storage: Store at room temperature, away from light in a solvent storage cabinet.

Fire and Explosion Data: Fire involving DCM is unlikely. In the event of fire use extinguishing media appropriate for surrounding fire.

Spill or Leak Procedures: Wear self-contained breathing apparatus and full protective clothing. Use water spray to reduce vapors. Take up with sand or vermiculite and place in closed container. Flush spill area with water.

Iodine

Iodine is extremely destructive to the tissue of the mucous membranes and upper respiratory tract, eyes and skin. Inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema. Symptoms of exposure may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea and vomiting.

First Aid: Remove contaminated clothing and shoes. Flush eyes or skin with plenty of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician. Discard contaminated clothing and shoes.

Recommended Storage: Keep tightly closed. Store in a cool dry place.

Fire and Explosion Data: Emits toxic fumes under fire conditions. Extinguish with dry chemical powder. Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Monomers (Amidites)

The amidite monomers should be considered hazardous as the toxicological properties are unknown. Do not breathe the powders.

Recommended Storage*:* Store at -20°C in a desiccator or sealed bag.

1-N-Methylimidazole

1-N-Methylimidazole is a toxic chemical which is harmful if swallowed, inhaled, or absorbed through the skin. Causes burns. Inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema. Symptoms of exposure may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea and vomiting.

First Aid: Remove contaminated clothing and shoes. Flush eyes or skin with plenty of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician. Wash contaminated clothing before reuse.

Recommended Storage: Hygroscopic solid. Handle and store under nitrogen. Keep tightly closed. Store in a cool dry place. Keep away from heat and open flame.

Fire and Explosion Data: Emits toxic fumes under fire conditions. Extinguish with CO_2, dry chemical powder, alcohol or polymer foam, water spray. Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Spill or Leak Procedures: Evacuate area. Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Absorb on sand or vermiculite and place in a closed container and hold for waste disposal.

Oxidizer Solution - Amidite Chemistry

This solution contains *iodine, pyridine, tetrahydrofuran (THF),* and water. See the individual reagents for details of safety precautions.

***Recommended Storage*:** Store at room temperature.

Pyridine

Pyridine is a flammable liquid with a characteristic disagreeable odor. Harmful if swallowed, inhaled, or absorbed through the skin. Causes severe irritation. High concentrations are extremely destructive to tissues of the mucous membranes and upper respiratory tract, eyes and skin. Symptoms of exposure may include burning sensation, coughing, wheezing, laryngitis, gastrointestinal disturbances. Chronic effects are damage to liver and kidneys.

First Aid: Remove contaminated clothing and shoes. Immediately flush eyes or skin with plenty of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Wash contaminated clothing before reuse.

Recommended Storage: Store in a cool dry place. When in use, handle and store under argon. Keep away from heat, sparks, and open flame.

Fire and Explosion Data: Flashpoint 20 °C. Extinguish with CO_2, dry chemical powder, alcohol or polymer foam. Water may be effective for cooling but may not effect extinguishment. Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. Vapor may travel considerable distance to source of ignition and flash back. Forms explosive mixture in air. Emits toxic fumes under fire conditions.

Spill or Leak Procedures: Shut off all sources of ignition. Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Cover with dry-lime, sand, or soda ash, place in covered containers using non-sparking told and transport outdoors. Ventilate area and wash spill site after material pickup is complete.

Reaction Columns

Recommended Storage: Store reaction columns dry, at 4°C and away from light.

Sulfurizing Reagent

The sulfurizing reagent used in amidite synthesis of phosphorothioates is 3H-1,2-benzodithiol-3-one, 1,1-dioxide.

***Recommended Storage*:** Contact supplier for information.

1H-Tetrazole

White powder and chips which may be harmful by inhalation, ingestion, or skin absorption. May cause irritation. The chemical, physical and toxicological properties of tetrazole have not been thoroughly investigated.

First Aid: Flush eyes with plenty of water for at least 15 minutes. Wash skin with soap and water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician. Wash contaminated clothing before reuse.

Recommended Storage: Keep tightly closed. Store in a cool dry place.

Fire and Explosion Data: Extinguish with water spray, CO_2, dry chemical powder, alcohol or polymer foam. Emits toxic fumes under fire conditions. Use water spray to cool fire-exposed containers. Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. Container explosion may occur under fire conditions.

Spill or Leak Procedures: Wear self-contained breathing apparatus, rubber boots and rubber gloves. Sweep up and place in a container. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Tetrahydrofuran (THF)

THF is a flammable solvent which will form explosive peroxides over a period of time in the presence of oxygen. It may be harmful by inhalation, ingestion, or skin absorption. Vapor or mist is irritating to the eyes, mucous membranes and upper respiratory tract. Causes skin irritation. Exposure can cause coughing, chest pains, difficulty in breathing, nausea, dizziness and headache.

First Aid: Remove contaminated clothing and shoes. Flush skin or eyes with plenty of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician. Wash contaminated clothing before reuse.

Recommended Storage: Hygroscopic, keep tightly closed. Store under nitrogen in a cool dry place. Keep away from heat, sparks, and open flame. Dispose of any opened Cap A or B bottles that are over six months old.

Fire and Explosion Data: Extremely flammable, vapor may travel considerable distance to source of ignition and flash back. Container explosion may occur under fire conditions. Extinguish with CO_2, dry chemical powder, alcohol or polymer foam. Water may be effective for cooling, but may not effect extinguishment. Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Spill or Leak Procedures: Evacuate the area. Shut off all sources of ignition. Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Cover with activated carbon adsorbent, place in covered containers using non-sparking tools and transport outdoors. Ventilate area and wash spill site after material pickup is complete.

Trichloroacetic Acid (TCA)

White crystals which are harmful if swallowed, inhaled, or absorbed through the skin. TCA is destructive to tissues of the mucous membranes and upper respiratory tract, eyes, and skin. Inhalation may be fatal as a result of spasm, inflammation and edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema. Symptoms of exposure may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea and vomiting. Causes blisters on contact with skin.

First Aid: Remove contaminated clothing and shoes. Flush eyes or skin with plenty of water for at least 15 minutes. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician. Wash contaminated clothing before reuse.

***Recommended Storage*:** Keep tightly closed. Store in a cool dry place.

***Fire and Explosion Data*:** Extinguish with CO_2, dry chemical powder, alcohol, or polymer foam. Emits toxic fumes under fire conditions. Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Spill or Leak Procedures: Evacuate the area. Shut off all sources of ignition and ventilate the area. Wear self-contained breathing apparatus, rubber boots and heavy gloves. Cover with dry lime or soda ash, scoop into a closed container and transport outside. Ventilate area and wash spill site after material pickup is complete.

C Performance Specifications

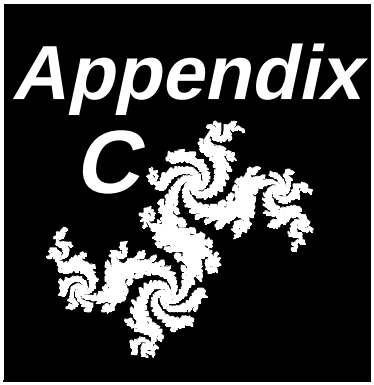


Table C-1 lists the specifications for the Expedite Nucleic Acid Synthesis System (Models 8909 and 8905).

Table C-1 Specifications for Expedite Nucleic Acid Synthesis System

		Model 8909	**Model 8905**
Reagent Handling	Number of amidite bottles	9	5
	Number of reagent bottles	6 internally plus 2 in an external tray	6
	Number of columns	2 independent	
Cycle time		<4 minutes in single and dual column mode (for up to 1 μmole DNA scale)	
Average stepwise coupling efficiency		≥98% by ion exchange HPLC (polyT) [31]	
Amidite consumption per cycle		<3 mg at 0.05 μmole	
Ancillary reagent consumption per cycle		<4.5 mL (for up to 1 μmole DNA scale)	
Maximum number of cycles per reagent kit		800	150

Table C-1 Specifications for Expedite Nucleic Acid Synthesis System (Continued)

		Model 8909	Model 8905
Bottle Sizes	Amidites	10 mL or 20 mL (0.5 mg or 1.0 mg)	5 mL (0.25 mg)
	WSH	300 mL or 450 mL	450 mL
	WSH A	2000 mL	N/A
	OX	200 mL or 450 mL	60 mL
	DBLK	900 mL	180 mL
	ACT	200 mL or 450 mL	60 mL
	CAP A	200 mL or 450 mL	60 mL
	CAP B	200 mL or 450 mL	60 mL
	AUX	200 mL	N/A
	Waste—chlorinated	1 Liter	
	Waste—organic	4 Liters	
Pulse volume		15.5 µL ±20%	
Pulse rate		2.8 to 4.5 pulses/second	
Blanket pressure		6 psi	
Inlet pressure		20 psi	
Gas purity (N, He, Ar)		high purity, 99.995%	
Drip tray volume		1.7 Liter	
Operating Temperature		20–30 °C	
Operating Humidity		<80%	

Table C-1 Specifications for Expedite Nucleic Acid Synthesis System (Continued)

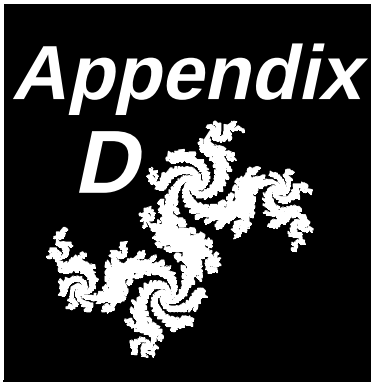
		Model 8909	Model 8905
Electronics	Power requirements	100–240 VAC, 47–63 HZ	
	Operational temperature range	4–40 °C	
Trityl Monitor	Type	monochrome light source	
	Wavelength	470 mm	
	Path length	2 nm	
	Flow cell volume	≥60 µL	
	Converter	12 bit A/D	
Control Module	User Interface	LCD—240x64 pixels 8 soft keys;1 fixed key LED indicator	
	Controller	68008 Microprocessor Clock Speed 5 MHZ EPROM 64 KB ROM 1 MB RAM	
	DRAM	1 MB	
	Nonvolatile RAM	50 bytes	
	External Connections	Parallel printer interface (25 pin “D” connector) RS-422 multidrop serial interface connection RS-422 serial interface connection 2 contact closures (external event) 1 switch closure input (UPS) 3.5” 1.44 MB floppy disk drive	

C

Table C-1 Specifications for Expedite Nucleic Acid Synthesis System (Continued)

		Model 8909	**Model 8905**
Pneumatics	Valve/solenoid driver	64 high current/voltage drivers	
	Max. current/voltage	350 mA per driver 24 V	
	Protection	Temperature Current Transient suppression	
	Fault indication	LED/TTL signal	
External Workstation	Minimum hardware requirements	386SX 16 MHz 100% IBM compatible 4 MB RAM (8 MB recommended) 60 MB hard disk VGA graphics	
	Software platform	Microsoft Windows ver. 3.1 DOS ver. 5.0 or higher	

D Nucleic Acid Synthesis Reagents



This appendix contains the part numbers for the reagents used on the Expedite Nucleic Acid Synthesis System.

Table D-1 Ancillary Reagents for Model 8905

Part Number	Short Name	Bottle Position	Label Description	Volume
GEN 089640	Wsh	1	Acetonitrile	450 mL
GEN 089690	Dblk	3	Deblock (TCA) Solution	180 mL
GEN 089680	Act	4	Activator Solution	60 mL
GEN 089650	Ox	2	Oxidizer Solution	60 mL
GEN 089610	Cap A	5	Cap A	60 mL
GEN 089620	Cap B	6	Cap B	60 mL
GEN 902005	Amidite Diluent			50 mL
GEN 089600	Reagent Kit (contains Wsh, Dblk, Act, Ox, Cap A, Cap B, Amidite Diluent)			

Table D-2 Ancillary Reagents for Model 8909

Part Number	Short Name	Bottle Position	Label Description	Volume
GEN 002006	Wsh A	7	Acetonitrile (External)	2500 mL
GEN 089860	Wsh	1	Acetonitrile (Internal)	300 mL
GEN 089865	Wsh	1	Acetonitrile (Internal)	450 mL
GEN 089890	Dblk	8	Deblock (TCA) Solution	900 mL
GEN 089880	Act	4	Activator Solution	200 mL
GEN 089885	Act	4	Activator Solution	450 mL
GEN 089850	Ox	2	Oxidizer Solution	200 mL
GEN 089855	Ox	2	Oxidizer Solution	450 mL
GEN 089810	Cap A	5	Cap A	200 mL
GEN 089815	Cap A	5	Cap A	450 mL
GEN 089820	Cap B	6	Cap B	200 mL
GEN 089825	Cap B	6	Cap B	450 mL
GEN 066870	Amidite Diluent			100 mL
GEN 089800	Reagent Kit (contains Wsh, Dblk, Act, Ox, Cap A, Cap B, Amidite Diluent)			

Table D-3 Expedite Amidites and Cap A for Model 8905

Part Number	Short Name	Label Description	Quantity
GEN 084154	Exp. dA	Expedite dA Cyanoethyl Phosphoramidite	0.5 g
GEN 084155	Exp. dC	Expedite dC Cyanoethyl Phosphoramidite	0.5 g
GEN 084156	Exp. dG	Expedite dG Cyanoethyl Phosphoramidite	0.5 g
GEN 066030	T	T Cyanoethyl Phosphoramidite	0.5 g
GEN 084280		Expedite Kit (contains A, C, G, T)	0.5 g x 4
GEN 084162	Exp. Cap A	Expedite Cap A	60 mL

Table D-4 Expedite Amidites and Cap A for Model 8909

Part Number	Short Name	Label Description	Quantity
GEN 084154	Exp. dA	Expedite dA Cyanoethyl Phosphoramidite	0.5 g
GEN 084155	Exp. dC	Expedite dC Cyanoethyl Phosphoramidite	0.5 g
GEN 084156	Exp. dG	Expedite dG Cyanoethyl Phosphoramidite	0.5 g
GEN 066030	T	T Cyanoethyl Phosphoramidite	0.5 g
GEN 084280		Expedite Kit (contains A, C, G, T)	0.5 g x 4
GEN 084151	Exp. dA	Expedite dA Cyanoethyl Phosphoramidite	1.0 g
GEN 084152	Exp. dC	Expedite dC Cyanoethyl Phosphoramidite	1.0 g
GEN 084153	Exp. dG	Expedite dG Cyanoethyl Phosphoramidite	1.0 g
GEN 066031	T	T Cyanoethyl Phosphoramidite	1.0 g
GEN 084281		Expedite Kit (contains A, C, G, T)	1.0 g x 4
GEN 084163	Exp. Cap A	Expedite Cap A	200 mL

Table D-5 Standard Beta-Cyanoethyl Phosphoramidite Monomers

Part Number	Name	Quantity
GEN 066000	DMT-dAdenosine (bz) Cyanoethyl Phosphoramidite	0.5 g
GEN 066010	DMT-dCytidine (bz) Cyanoethyl Phosphoramidite	0.5 g
GEN 066020	DMT-dGuanosine (ibu) Cyanoethyl Phosphoramidite	0.5 g
GEN 066030	DMT-Thymidine Cyanoethyl Phosphoramidite	0.5 g
GEN 066040	Phosphoramidite Kit (contains A, C, G, and T)	0.5 g x 4
GEN 066050	DMT-dInosine Cyanoethyl Phosphoramidite	0.25 g
GEN 066060	DMT-dUridine Cyanoethyl Phosphoramidite	0.25 g
GEN 066070	DMT-5- Methyl dCytidine (bz) Cyanoethyl Phosphoramidite	0.25 g
GEN 066001	DMT-dAdenosine (bz) Cyanoethyl Phosphoramidite	1.0 g
GEN 066011	DMT-dCytidine (bz) Cyanoethyl Phosphoramidite	1.0 g
GEN 066021	DMT-dGuanosine (ibu) Cyanoethyl Phosphoramidite	1.0 g
GEN 066031	DMT-Thymidine Cyanoethyl Phosphoramidite	1.0 g
GEN 066041	Phosphoramidite Kit (contains A, C, G, and T)	1.0 g x 4

Table D-6 Pre-Packed Columns for Beta-Cyanoethyl Phosphoramidite Synthesis

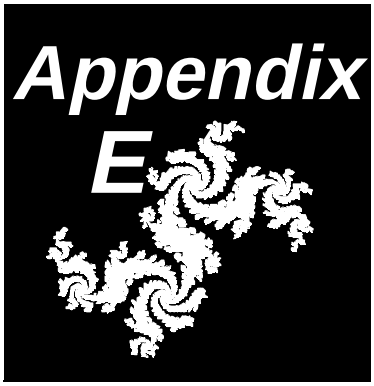
Description	Synthesis Scale	Part Number		Cols/Pkg
		500 A	1000 A	
DMT-dAdenosine (bz)-CPG	0.05 μmol	GEN 062700	-	4
	0.2 μmol	GEN 062650	GEN 062600	4
	1.0 μmol	GEN 061550	GEN 061500	4
	15 μmol	GEN 061750	-	1
DMT-dCytidine (bz)-CPG	0.05 μmol	GEN 062710	-	4
	0.2 μmol	GEN 062660	GEN 062610	4
	1.0 μmol	GEN 061560	GEN 061510	4
	15 μmol	GEN 061760	-	1
DMT-dGuanosine (ibu)-CPG	0.05 μmol	GEN 062720	-	4
	0.2 μmol	GEN 062670	GEN 062620	4
	1.0 μmol	GEN 061570	GEN 061520	4
	15 μmol	GEN 061770	-	1
DMT-Thymidine-CPG	0.05 μmol	GEN 062730	-	4
	0.2 μmol	GEN 062680	GEN 062630	4
	1.0 μmol	GEN 061580	GEN 061530	4
	15 μmol	GEN 061780	-	1
Column Kit contains one column each of A, C, G, and T	0.05 μmol	GEN 062740	-	4
	0.2 μmol	GEN 062960	GEN 062640	4
	1.0 μmol	GEN 061590	GEN 061540	4

Table D-7 Pre-Packed Expedite Columns

Description	Synthesis Scale	Part Number		Cols/Pkg
		500 A	1000 A	
dAdenosine tBPA-CPG	50 nmol	GEN 084164	-	4
	0.2 µmol	GEN 084167	GEN084179	4
	1.0 µmol	GEN084170	GEN084182	4
dCytidine tBPA-CPG	50 nmol	GEN 084165	-	4
	0.2 µmol	GEN 084168	GEN084180	4
	1.0 µmol	GEN084171	GEN084183	4
dGuanosine tBPA-CPG	50 nmol	GEN084166	-	4
	0.2 µmol	GEN084169	GEN084181	4
	1.0 µmol	GEN084172	GEN084184	4
Thymidine-CPG	50 nmol	GEN062730	-	4
	0.2 µmol	GEN062680	GEN062630	4
	1.0 µmol	GEN061580	GEN061530	4
50 nmol Column kit (contains one 50 nmol column of each A, C, G, and T)	50 nmol	GEN084296	-	4
0.2 µmol Column kit (contains one 0.2 µmol column of each A, C, G, and T)	0.2 µmol	GEN084298	GEN084303	4
1.0 µmol Column kit (contains one 1.0 µmol column of each A, C, G, and T)	1.0 µmol	GEN084300	GEN084305	4

Table D-8 MemSyn™ Nucleic Acid Synthesis Devices

Part Number	Description
GEN 050006	MemSyn A, 50 nmol scale
GEN 050016	MemSyn C, 50 nmol scale
GEN 050026	MemSyn G, 50 nmol scale
GEN 050036	MemSyn T, 50 nmol scale
GEN 050004	MemSyn A, 0.2 µmol scale
GEN 050014	MemSyn C, 0.2 µmol scale
GEN 050024	MemSyn G, 0.2 µmol scale
GEN 050034	MemSyn T, 0.2 µmol scale



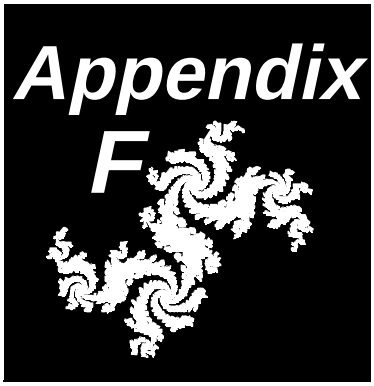
E Accessories and Spare Parts

This appendix contains the part numbers of the accessories and spare parts for the Expedite Nucleic Acid Synthesis System.

Accessories	GEN 210222	Uninterruptable Power supply (115 Vac) kit w/cable
	GEN 210282	Uninterruptable Power supply (220 Vac) kit w/cable
	GEN 210219	Cable, Frac. Col., ISCO, 8900
	GEN 103258	Gas tank regulator, kit
Miscellaneous	GEN 210131	Drip tray, chassis, replacement
	GEN 210147	Fuse, 2A, 5x20mm, 2/pk
	GEN 210208	RS422 Interface card, AT style
	GEN 210212	Adapter, DB-25M to RJ45
	GEN 210209	Cable, interconnect, RJ45 flat, 14 ft.
Replacement Filters	GEN 210168	Kit, Reservoir. filter, 8909 (19/pk)
	GEN 210169	Kit, Reservoir. filter, 8905 (12/pk)

Replacement 0-rings	GEN 210164	Kit, O-ring, Rgt. Res., 8905/09
	GEN 210165	Kit, O-ring, Nucl. Res, 8909
	GEN 210166	Kit, O-ring, Nucl. Res, 8905
	GEN 210167	Kit, O-ring, Caddy, 8909
	GEN 317081	O-ring, for 38mm, cap,10/ pk
Replacement Reservoirs	GEN 105915	Bottle, 4L, amber, plastic-coated (Waste)
	GEN 330220	Bottle, 1L, plastic coated, (External Deblock, Trityl Waste)
	GEN 102461	Bottle, 2L, plastic coated (Wash)
	GEN 322140	Bottle, boston round, 1 oz (Monomers)
	GEN 322150	Bottle, boston round, 6 oz (Reagents), 8905 only
	GEN 332160	Bottle, boston round, 8 oz (Reagents)
Options	GEN 210182	Expedite Workstation software
	GEN 600173	Multiple Oligonucleotide Synthesis System (MOSS option)
	GEN 600175	Expedite PNA Instrument option
Documentation	601318	Documentation set containing *Expedite 8900 Nucleic Acid Synthesis System User's Guide* and *Expedite 8900 Nucleic Acid Synthesis System Quick Reference Card*
	601307	*Multiple Oligo Synthesis System (MOSS) for the Expedite 8900 Nucleic Acid Synthesis System User's Guide*
	601308	*PNA Chemistry for the Expedite 8900 Nucleic Acid Synthesis System User's Guide*
	601309	*Expedite 8900 Nucleic Acid Synthesis System Macintosh Interface User's Guide*

F Trityl Monitor



This appendix contains the following sections:

F.1 Introduction

The Expedite Trityl Monitor detects the acid-labile dimethoxy trityl (DMT) protecting group removed from the 5' end of an oligonucleotide during the deblocking step in the synthesis. The amount of DMT released from the column is an indication of the success of the addition of the last monomer to the growing oligonucleotide chain. The DMT group is an orange species in the acidic deblocking solution which the trityl monitor is tuned to detect as it passes through a flow cell. There is a photo detector on the trityl monitor board and an LED on the opposite side of the flow cell which emits light at 470nm, a wavelength which DMT absorbs.

NOTE: *Helium is the recommended gas when an instrument is equipped with a trityl monitor because helium produces fewer bubbles during deblocking. Gas bubbles passing through the trityl monitor may interfere with its function.*

Service

The trityl monitor must be serviced by trained PerSeptive Biosystems personnel. Do not attempt to service the trityl monitor yourself. Contact your local PerSeptive Biosystems representative.

Display

During deblocking, the orange DMT solution passes through a flow cell. When deblocking is complete, the software displays the processed detector output as a bar chart to allow you to monitor the progress of each coupling cycle. The instrument screen can display trityl monitor determinations for up to 250 couplings.

NOTE: *Use trityl monitor determinations only as approximations of the success rate of couplings and for estimating total yield. They are not a substitute for quantitative analysis.*

The trityl monitor is mounted inside the instrument cabinet behind the columns and is inaccessible to the user. If a leak occurs, the integral drip tray drains to the column drip tray which in turn drains into the instrument drip tray.

If the trityl monitor detects a total yield failure (when the yield from a deblock step has fallen below a minimum threshold), it will, at the option of the user, halt the synthesis at the end of the current cycle. When you do not want to use this feature, disable the trityl alarm for Column1, Column 2 or both. See Section F.5, Trityl Monitor Alarms, for details.

Waste Fluids

The trityl monitor has a diversion valve to direct chlorinated and nonchlorinated wastes to separate waste containers for ease of disposal. There are four waste lines:

- Column 1 chlorinated waste
- Column 1 nonchlorinated waste
- Column 2 chlorinated waste
- Column 2 nonchlorinated waste

DMT waste is included in the chlorinated waste effluent.

Four bands identify each waste tube. Waste tube band markings are summarized in Table F-1. The chlorinated waste lines can also be connected to a fraction collector to gather the trityl color for visual inspection.

Table F-1 Waste Tube Band Markings

Column Number	Type of Waste	Waste Tube Band Markings
1	Chlorinated	T R T 1
	Nonchlorinated	O R G 1
2	Chlorinated	T R T 2
	Nonchlorinated	O R G 2

Fraction collector

There are two event outputs (contact closures) at the back of the synthesizer that can be used to advance a fraction collector: one (Event 1) for Column 1 and one (Event 2) for Column 2. Consult the technical manual of the fraction collector for connection instructions.

F.2 Safety Precautions

The following information is provided to ensure safe operation of the Expedite Nucleic Acid Synthesis System. Please read it carefully before using the instrument and observe the following safety recommendations.

Electrical

Use only the shielded AC cord provided. You must unplug the main power cable before you service or repair the instrument.

WARNING

To prevent electric shock, do not remove the instrument cover. There are no user serviceable parts within the cabinet. Refer servicing to qualified personnel.

AVERTISSEMENT

Afin d'éviter les chocs électriques, ne retirez pas le couvercle de l'appareil. Il n'y a à l'intérieur de l'appareil aucune pièce que vous puissiez remplacer vous-même. Faites appel au service technique de PerSeptive Biosystems.

Gas Supply

Securely anchor the tank of pressurized gas.

We recommend the use of a dual stage regulator to step the pressure down to 20 psi.

Operating Temperature

The protocols are optimized for room temperature operation (20–30°C).

Instrument Venting

The instrument cabinet is equipped with an exhaust fan that vents vapors to the rear of the instrument. Make sure that the vent tube on the back of the instrument is connected to a suitable exhaust duct.

Reagent Handling

Most of the reagents and solvents used in nucleic acid synthesis are hazardous. Wear a lab coat, gloves and eye protection when handling reagents. Adequate ventilation is essential and working under a fume hood is recommended.

Flammable reagents should be kept in an appropriate flameproof cabinet. Oxidizers should be isolated. Consult the *Expedite 8900 User's Guide*, Appendix B, for information on the handling of specific chemicals.

Make sure that you put the reagent bottles on the instrument in the correct position. Mixing up the bottles will contaminate the instrument. The reagent bottles on the instrument are pressurized. Take care when removing the bottles from the instrument.

Waste Disposal

Collect the waste generated from oligonucleotide synthesis and dispose of it in accordance with local, state, and federal regulations pertaining to toxic waste removal.

F.3 Displaying Trityl Data

Use the following procedure to display trityl monitor data.

1. If the Main menu is not displayed, press **Exit** as many times as necessary to display the Main menu shown in Figure F-1.

Figure F-1 Main Menu

NOTE: *Previous trityl data is only retained until another synthesis is run, except when the instrument is communicating with an Expedite Workstation.*

2. From the Main menu select **Stat** to display the Status: Combined menu shown in Figure F-2.

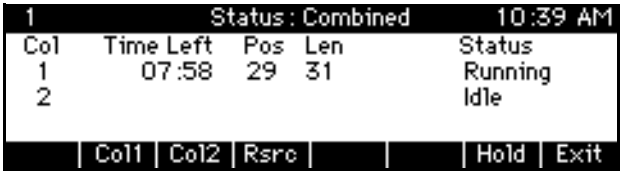


Figure F-2 Status: Combined

F

3. From the Status: Combined menu select **Col1** or **Col2** to display the Status: Column # menu shown in Figure F-3.

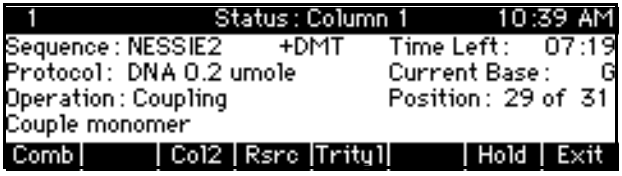


Figure F-3 Status: Column # Menu

NOTE: *The number sign (#) is either 1 or 2 depending on whether Column 1 or Column 2 is selected.*

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4. From the Status: Column # screen select **Trityl** to display the Status: Trityl Col # screen shown in Figure F-4. The system automatically updates the bar graph as the synthesis progresses.

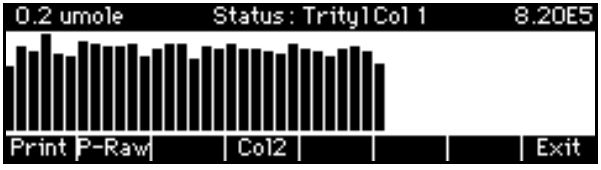


Figure F-4 Status: 11Trityl Col # Screen

The number in the upper right corner of the screen represents the value for the highest bar on the histogram. This number is unitless and represents the raw integration value of the maximum bar (excluding the 3' bar).

NOTE: *Do not use this number to quantify the performance of the synthesizer. This number is only a qualitative tool for estimating the synthesis scale and for troubleshooting.*

F

The number in the upper left corner of the screen is *only an estimate of the synthesis scale*. The scale estimation is based on the integration value of the highest bar in the graph (excluding the first bar) assuming that standard PerSeptive Biosystems protocols are used for the synthesis.

Possible values for the estimated scale are listed in Table F-2.

Table F-2 Trityl Viewer Scale Estimates

Scale	Explanation
Low 0.02 µmole	• The estimated synthesis scale is less than 0.05 µmole. • There may be a problem with the synthesis or the trityl monitor (see Section F.4, Interpreting Trityl Data)
0.05 µmole	The estimated synthesis scale is within the 0.05 µmole range.
0.2 µmole	The estimated synthesis scale is within the 0.2 µmole range.
1.0 µmole	The estimated synthesis scale is within the 1.0 µmole range.
15 µmole	The estimated synthesis scale is within the 15 µmole range.
High	• The estimated synthesis scale is greater than 15 µmole. • There may be a problem with the synthesis or the trityl monitor (see Section F.4, Interpreting Trityl Data)

5. To view the trityl data on the other column, select **Col#**.

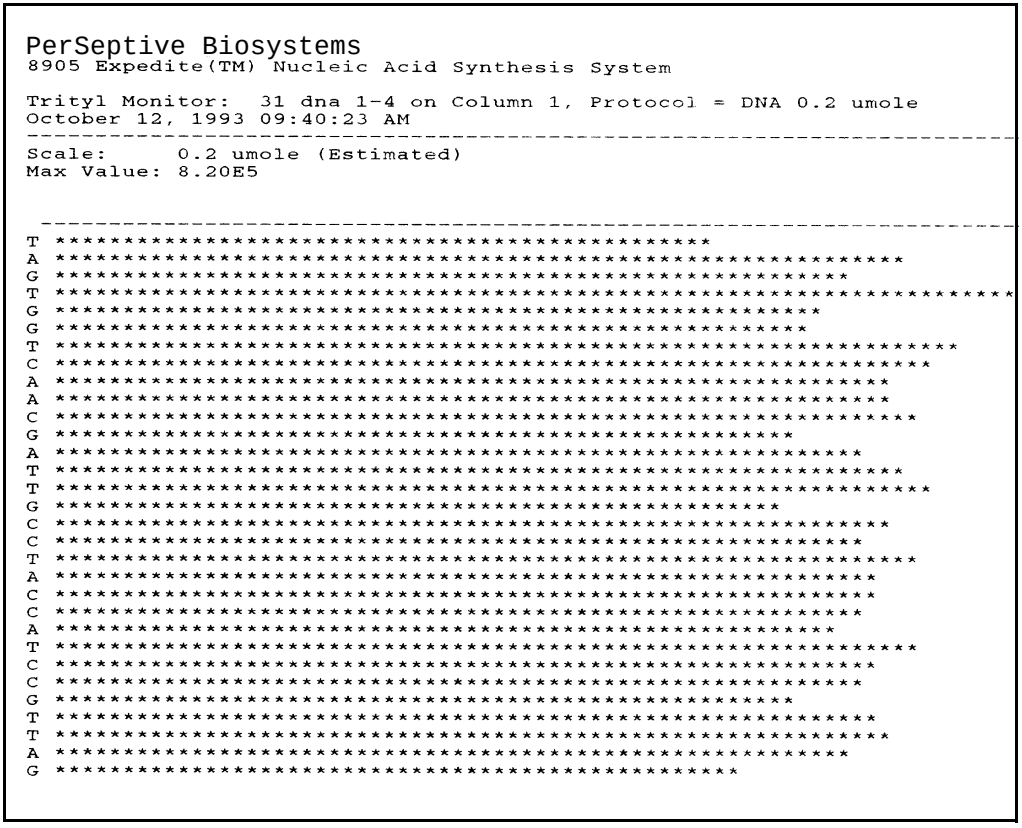
Printing Trityl Data

Trityl data may be printed out on a printer connected to the printer port on the instrument; or captured by the Expedite Workstation Software.

To obtain a printed copy of trityl monitor data, select either of the following options:

- **Print** provides a printout similar to that shown in Figure F-5.
- **P-raw** provides a printout that includes the raw data values.

NOTE: *Use trityl monitor determinations only as approximations of the success of couplings and for estimating synthesis scale. These values are not a substitute for quantitative analysis.*



```
PerSeptive Biosystems
8905 Expedite(TM) Nucleic Acid Synthesis System

Trityl Monitor:  31 dna 1-4 on Column 1, Protocol = DNA 0.2 umole
October 12, 1993 09:40:23 AM
-------------------------------------------------------------------------------
Scale:      0.2 umole (Estimated)
Max Value: 8.20E5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T ***********************************************
A ************************************************************
G ***********************************************************
T ********************************************************************************
G ****************************************************
G ********************************************************
T ****************************************************************
C ***************************************************************
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Figure F-5 Trityl Data Printout

F

F.4 Interpreting Trityl Data

Data from the trityl monitor is displayed in a bar graph format. Each bar, which is an indication of the success of the previous coupling, represents the cumulative photometric data collected from the trityl monitor during the deblocking step. Individual bars in the graph may be wider or narrower than the examples shown here depending on the number of couplings displayed.

NOTE: *Use trityl monitor determinations only as approximations of the success of couplings and for estimating synthesis scale. These values are not a substitute for quantitative analysis.*

Normal Synthesis

A normal synthesis, shown in Figure F-6, typically shows an overall decline in the height of the bars as each monomer is added.

The 3' reading (i.e., the leftmost bar of the graph) is ignored for scale estimation and trityl alarms because:

- Gas trapped in the newly mounted column may interfere with the trityl monitor's functioning resulting in an abnormal reading.
- The DMT protecting group of the 3' monomer attached to the column may have been lost while in storage.

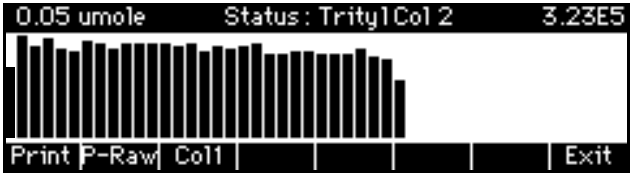

Figure F-6 Normal Synthesis

NOTE: *Always store columns in a cool desiccator to prevent spontaneous loss of the DMT protecting group.*

The overall appearance of the bar graph may appear ragged. This irregularity can be caused by:

- Deblock solution out-gassing within the flow cell.
- The DMT deblocking rate differences for various monomers. Typically the DMT group is removed from a G residue in the shortest period of time which results in a more intensely colored solution. Conversely, the DMT group is removed from a T residue in the longest period of time which results in the least intensely colored solution. Because the absorbance intensity is not linearly proportional to the concentration of DMT, the amount of DMT is underestimated for a G residue and is most representative for a T residue. Therefore, the bar for a G residue is somewhat smaller than the average and the bar for T residues is somewhat larger than average (see Figure F-5).
- Electronic noise or ordinary drift in the trityl monitor.

Initial Failure

An initial failure is displayed by the trityl monitor as a synthesis in which the bar size quickly drops to a level which is unacceptably low (see Figure F-7).

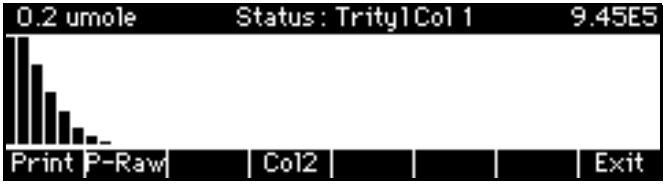

Figure F-7 Failed Synthesis

If trityl monitor alarms are enabled (see Section F.5, Trityl Monitor Alarms), a total yield failure may occur and the synthesis would then be halted at the end of the current cycle.

A synthesis can fail because:

- The reagents have degraded
- The reagents are not anhydrous
- Low delivery of monomer or activator (Reservoir is empty)
- Inefficient deblocking

Sudden Failure

A sudden failure is one in which there is a drastic decrease in bar size after initial successful couplings (see Figure F-8). A sudden failure can occur because:

- A reagent bottle may be depleted of solution
- A pneumatic failure may have occurred (over or under pressure, leaking gas or running out of gas)

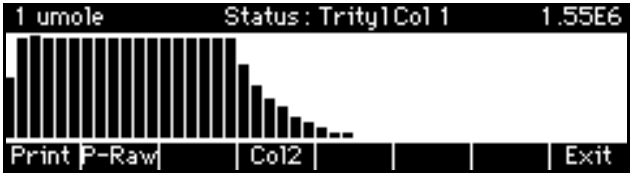

Figure F-8 Drastic Failure

If trityl monitor alarms are enabled (see Section F.5, Trityl Monitor Alarms), a total yield failure may occur and the synthesis would then be halted at the end of the current cycle.

NOTE: *A pneumatic failure would be detected by sensors in the system unless they are disabled.*

F

System Failure

A system failure is a failure caused by an instrument malfunction. System failures may produce a trityl display such as that shown in Figure F-9.

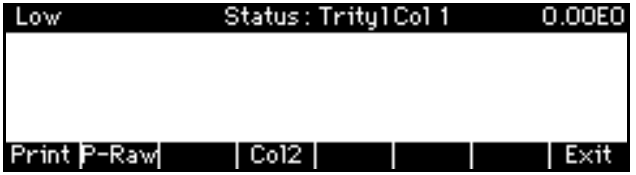

Figure F-9 System Failure

A system failure could be caused by one of the following:

- An electronic failure. (Contact your PerSeptive Biosystems Service Representative.)
- A problem with the trityl monitor optics. (Contact your PerSeptive Biosystems Service Representative.)

Delivery Failure

A delivery failure may be caused by the failure of fluid delivery through the reaction column. This type of failure results in random trityl monitor displays as shown in Figure F-10.

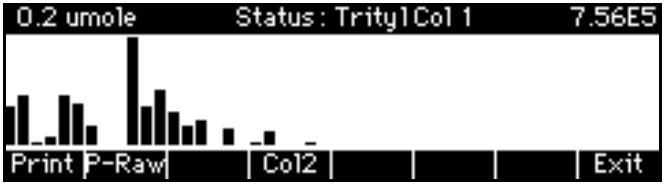

Figure F-10 Delivery Failure

F

A fluid delivery failure can be caused by any of the following:

- A pneumatic failure (running out of gas).
- A blockage in the flow path (check waste tubing).
- Fluid lines from the columns to the trityl monitor are switched (that is, column 1 output goes into the column 2 of the trityl monitor flow cell, or the reverse).

F.5 Trityl Monitor Alarms

This section describes the halting of a synthesis by the trityl monitor and how to proceed when the trityl monitor signals a total yield failure. It also describes how to enable and disable trityl monitor alarms.

Halted Synthesis

When the trityl monitor detects a total yield failure, the system displays the screen shown in Figure F-11. If the trityl monitor alarm is enabled, it halts the synthesis at the end of the current cycle.

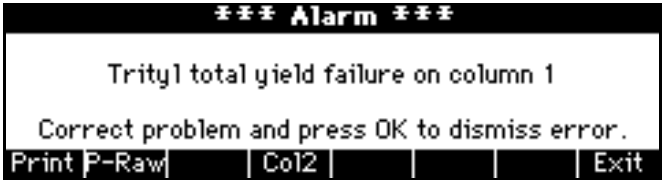

Figure F-11 Trityl Total Yield Failure Alarm

When a synthesis has been halted, perform the following operations.

1. Press **OK** to acknowledge the alarm. The display returns to the screen displayed before the alarm.
2. See Section F.4, Interpreting Trityl Data, and determine the nature of the problem that caused the alarm.
3. Correct the problem.

4. Determine whether you want the synthesis to continue.
    - If you want the synthesis to continue:
        - If the Main menu is not displayed, press **Exit** as many times as necessary to display the Main menu.
        - From the Main menu press **Start** to continue the synthesis.
    - If the synthesis has been compromised and you want to abort:
        - If the Main menu is not displayed, press **Exit** as many times as necessary to display the Main menu.
        - From the Main menu choose **Seq** to display the Sequence menu.
        - From the Sequence menu choose **Abort** to terminate the synthesis. The Main menu will then be displayed.

If you do not want a synthesis halted by a trityl monitor alarm, change the profile to disable the alarm.

NOTE: *If the trityl total yield failure alarm is displayed and the synthesis has not yet come to the end of the current cycle, pressing* ***OK*** *will cause the synthesis to continue without halting.*

Enabling/ Disabling the Alarms

The trityl monitor alarms are enabled by default for every profile. To customize the alarms (that is to turn them on or off for a particular profile), use the following procedure.

1. If the Main menu is not displayed, press ***Exit*** as many times as necessary to display the Main menu shown in Figure F-12.

Figure F-12 Main Menu

2. If the profile number displayed in the Main menu is the one whose alarms you want to enable or disable, go to step 3. If the profile number is not the one you want to work with, select the desired profile number. See Section 3.6.6, Specifying a User Profile, for information on changing profiles.

3. From the Main menu select **Tools** to display the Tools menu shown in Figure F-13.

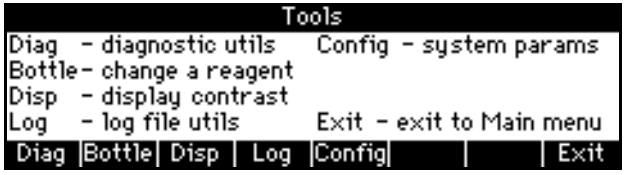

Figure F-13 Tools Menu

F

4. From the Tools menu select **Config** to display the Tools: Config menu shown in Figure F-14.

Figure F-14 Tools: Config Menu

5. From the Tools: Config menu select **Profil** to display the Tools—Config: Profile # menu shown in Figure F-15.

Figure F-15 Tools—Config: Profile # Menu

NOTE: *The number sign (#) will be 1–8 depending on the number of the profile.*

F

6. From the Tools—Config: Profile # menu select **Edit** to display the first profile configuration screen shown in Figure F-16.

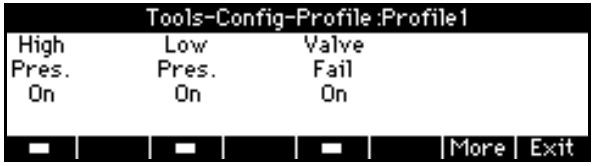

Figure F-16 Tools—Config—Profile: Profile # Menu

7. Select **More** to display the second profile configuration screen shown in Figure F-17.

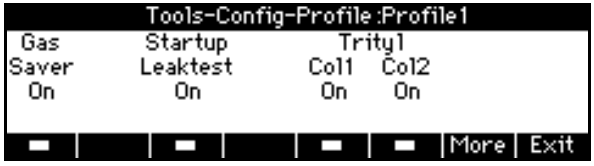

Figure F-17 Tools—Config—Profil: Alarm Menu

8. Press the soft keys to toggle the trityl alarms **On** and **Off**.

   - **Trityl Col1 On** or **Off**
   - **Trityl Col2 On** or **Off**

   The factory default settings for the trityl monitor alarms are shown in Figure F-17.

The trityl monitor alarm functions that are assigned to the soft toggle keys are described in Table F-3.

Table F-3 Trityl Monitor Alarms

Key #	Key Name	Function
5	Trityl Col1	To enable or disable the trityl monitor alarm for Column1.
6	Trityl Col2	To enable or disable the trityl monitor alarm for Column2.

9. From the Tools—Config—Profile: Profile # menu select **Exit** to display the *User Profile: Profile #* screen shown in Figure F-18.

Figure F-18 User Profile: Profile # Screen

10. Select **OK** to record trityl monitor alarm setting changes in the designated profile.

F

F.6 Troubleshooting

If the following conditions arise in the ordinary use of a system with the trityl monitor installed, proceed as directed below.

- The system produces the “No trityl monitor installed” messages shown in Figure F-19 when the trityl monitor is, in fact, installed. Contact your PerSeptive Biosystems Service Representative.

Figure F-19 No Trityl Monitor Detected Screen

- Orange fluid is observed in the column drip tray or in the removable instrument cabinet drip tray. Make sure the column is installed correctly. If orange fluid continues to appear, contact your PerSeptive Biosystems Service Representative.
- The trityl monitor indicates a system failure. See Section F.4, Interpreting Trityl Data. Contact your PerSeptive Biosystems Service Representative.
- Fluid is not observed dripping into the trityl waste reservoir during the deblock step may indicate diversion valve failure. Contact your PerSeptive Biosystems Service Representative.

WARNING

An orange color in the drip tray may indicate leakage from the trityl monitor.

F

AVERTISSEMENT

Une couleur orange au niveau du plateau de récupération peut indiquer une fuite du moniteur de trityle.

There are no user corrective or preventive maintenance procedures for a system with a trityl monitor beyond those already discussed in the *Expedite 8900 Nucleic Acid Synthesis System User's Guide*.

G Warranty/Service Information

G.1 Limited Product Warranty

Warranty period

PerSeptive Biosystems, Inc. warrants that all standard components of its new instrument workstations (excluding items replaceable by the user, such as lamps, flow cells, seals, fittings, tubing, and filters) will be free of defects in materials and workmanship for a period of one (1) year. PerSeptive 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 PerSeptive at its published rates. PerSeptive Biosystems also provides service agreements for post-warranty coverage.

PerSeptive Biosystems, Inc., guarantees all optional accessories supplied with its instrument workstations, such as detectors, fraction collectors, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days. PerSeptive will repair or replace, at its discretion, all defective accessories during the warranty period. After this warranty period, PerSeptive 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.

PerSeptive Biosystems, Inc., warrants that all user-replaceable items, including lamps, flow cells, fittings, tubing, and filters are free of defects in materials and workmanship when received by the buyer, but not thereafter. PerSeptive also warrants all deuterium lamps to be free of defects in materials and workmanship and to operate above one half (1/2) of the original light intensity for a period equal to the lesser of one year or 1,000 hours of use. PerSeptive will repair or replace, at its discretion, all defective replaceable items during the applicable warranty period.

PerSeptive Biosystems, Inc. warrants that the software designated for use with the instrument will execute its programming instructions when properly installed on the product. PerSeptive does not warrant that the operation of the instrument or software will be uninterrupted or error free. PerSeptive Biosystems will provide any software corrections or “bug-fixes”, if and when they become available, for a period of one (1) year after installation.

Warranty period effective date

Any applicable warranty period under these sections will begin on the date of installation for hardware and software installed by PerSeptive personnel, unless that date has been delayed at the buyer's request. In that case, and for all hardware and software installed by the buyer, the applicable warranty period begins the date the component is received.

Warranty exceptions

The above warranties shall not apply to defects resulting from misuse, negligence, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental specifications of the instrument or accessories; performance of improper or inadequate maintenance by the user; installation of software or interfacing not supplied by PerSeptive; and modification of the instrument or the software not authorized by PerSeptive.

The foregoing provisions set forth PerSeptive's sole and exclusive representations, warranties, and obligations with respect to the products, and PerSeptive makes no other warranty of any kind whatsoever, express or implied, 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. Such limited warranty is given only to buyer of any third party in the event of use of products furnished hereunder by any third party.

Warranty limitations

The remedies provided herein are buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall PerSeptive be liable, whether in contact, in tort, or on any other basis, for direct, indirect, punitive, incidental, consequential, or special damages sustained by the buyer or any other person, whether or not foreseeable and whether or not PerSeptive is advised of the possibility of such damage, including without limitation, damage 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.

G.2 Damages, Claims, Returns

Damages

Please unpack any shipments promptly after receipt to check for any concealed damaged.

If you discover damage, stop unpacking. Contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support the claim. Do not return damaged goods to PerSeptive Biosystems without first securing an inspection report, and contacting PerSeptive Biosystems Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is secured, PerSeptive Biosystems will supply replacements and process claims which are initiated by either party.

Returns

Please do not return any material without prior notification and authorization.

If, for any reason, it becomes necessary to return material to us, please contact PerSeptive Biosystems Technical Support, or your nearest PerSeptive 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 appropriate address.

Index

C

D

E

F

G

H

I

L

M

N

O

P

R

S

T

U

V

W