COBRA AFC 2000

Robotic Sample Handling Device

User Guide

Applied Biosystems

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

POROS products and perfusive chromatography are covered by U.S. patents 5,552,041; 5,605,623; and 5,833,861; foreign patents pending. Chromatography materials of certain pore geometries, including certain wide-pore supports, can permit perfusive chromatography, that is, separations in which the rate of intraparticle convection exceeds the rate of intraparticle diffusion. Use of any such chromatography media at sufficiently high linear velocity, without license, may constitute patent infringement. A limited license to use the patented perfusive chromatography process is granted with the purchase of POROS products from Applied Biosystems. The license terminates upon expiration of the useful life of the product.

Printed in the USA, 08/2001
Part Number 8-0189-40-0597 Rev. A

Table of Contents

How to Use This Guide

Purpose Applied Biosystems *COBRA™ AFC 2000 Robotic Sample Handling Device User's Guide* describes the procedures for installing, running, maintaining, and troubleshooting the COBRA™ AFC 2000 Robotic Sample Handling Device. The guide also describes how to use the On Board Analysis™ software features with the AFC 2000.

Audience This guide is intended for novice and experienced BioCAD 700E Workstation users who develop and execute analytical and preparative separation protocols of biomolecules based on POROS® Perfusion Chromatography® media and conventional chromatography media.

Structure Applied Biosystems *COBRA AFC 2000 Robotic Sample Handling Device User's Guide* is divided into six 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, Introducing the COBRA™ AFC 2000 Robotic Sample Handling Device	Describes features and parts of the AFC 2000 Robotic Sample Handling Device.
Chapter 2, Connecting the AFC 2000 Robotic Sample Handling Device	Provides procedures for assembling and connecting the AFC 2000 to the BioCAD 700E Workstation.
Chapter 3, Using the AFC 2000 Robotic Sample Handling Device	Describes how to prepare, configure, and use the AFC 2000 Robotic Sample Handling Device to collect fractions and make injections.

Chapter 4, Using Automated Analysis	Explains how to use the Automated Analysis feature. Automated Analysis controls the AFC 2000 Robotic Sample Handling Device to automatically reinject collected fractions for specific analyses.
Chapter 5, Maintenance	Provides maintenance procedures for the AFC 2000 Robotic Sample Handling Device.
Chapter 6, Troubleshooting and Error Codes	Includes troubleshooting information and error codes related to the AFC 2000 Robotic Sample Handling Device.
Appendix A, Specifications	Includes specifications for the AFC 2000 Robotic Sample Handling Device.
Appendix B, Spare Parts	Provides a list of spare parts for the AFC 2000 Robotic Sample Handling Device.
Appendix C, Warranty/Service Information	Includes warranty information.
Appendix D, BioCAD Sample Methods	Describes the sample methods supplied with the system related to the Automated Analysis feature.
Appendix E, Technical Support and Training	Describes how to contact Technical Support, obtain technical documents, and obtain customer training information.

Conventions

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

General conventions

The following general conventions are used:

- **Bold** text 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:

  "Prior to operation, *always* check for eluent miscibility."

Notes, Hints, Cautions, and Warnings

Notes, Hints, Cautions, and Warnings are used as follows:

- A note calls out important information to the user:

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

- A hint provides helpful suggestions not essential to the use of the product:

  Hint: *Set the high pressure limit to the pressure limit of the column with the lowest pressure limit.*

- A caution calls out information to avoid damage to the system or equipment:

  CAUTION
  Do not touch the flow cell window. This may damage the flow cell.

- A warning calls out information essential to the safety of the user:

  WARNING
  Always observe safe laboratory practices when operating your system.

Remarques, recommandations et avertissements

Une remarque fournit une information importante à l'opérateur er se présente ainsi:

REMARQUE: *Si on vous demande d'insérer la disquette de démarrage dans le lecteur, insérez-la puis appuyez sur n'importe quelle touche.*

Une recommandation fournit une information destinée à éviter des détériorations du système et de la synthèse ou la perte de données:

RECOMMANDATION

La lampe peut être endommagée. N'y touchez pas.

Un avertissement fournit une information indispensable à la sécurité de l'operateur et se présente ainsi:

AVERTISSEMENT

Conformez-vous toujours aux règlements du laboratoire quand vous utilisez votre système.

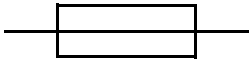
Symbols used on the system

The following symbol appears next to the AFC 2000. Keep hands out of the rack area when the AFC 2000 is in use.

Le symbole ci-contre figure à côté de l'AFC 2000. Veuillez garder les mains en dehors du bâti lorsque le AFC 2000 est utilisé.

The following symbol appears next to the values of the fuses required by the system.

Le symbole ci-contre figure à côté de la valeur des fusibles nécessaires avec ce système.



Related documentation

Use these documents in conjunction with the AFC 2000:

- Applied Biosystems ***BioCAD® 700E Workstation User's Guide*** —Use this guide to learn how to install, run, maintain, and troubleshoot your BioCAD 700E Workstation.
- Applied Biosystems ***BioCAD® 700E Workstation Getting Started Guide*** —Use this guide to learn the basics of operating your BioCAD 700E Workstation. It provides step-by-step information for running your first experiment.

Introducing the COBRA™ AFC 2000 Robotic Sample Handling Device

1

This chapter includes the following sections:

1

1.1 Overview

Uses

The COBRA™ AFC 2000 Robotic Sample Handling Device (Figure 1-1) allows you to conduct Chromatographic On Board Robotic Analyses.

You can use the COBRA AFC 2000 for:

- Integrated fraction collection
- Integrated autosampling
- Combined integrated fraction collection/autosampling
- Automated Analysis (automates purification and analysis)

Before you begin

To use the AFC 2000:

- Connect the AFC 2000 to the BioCAD 700E Workstation
- Prepare the AFC 2000
- Configure and prepare the BioCAD software for an AFC 2000

Controlling

After you complete the previous steps, you can use the AFC 2000 to collect fractions and make injections. You can control the AFC 2000 in two ways:

- From the Sample Table and Control Panel
- By creating and running methods

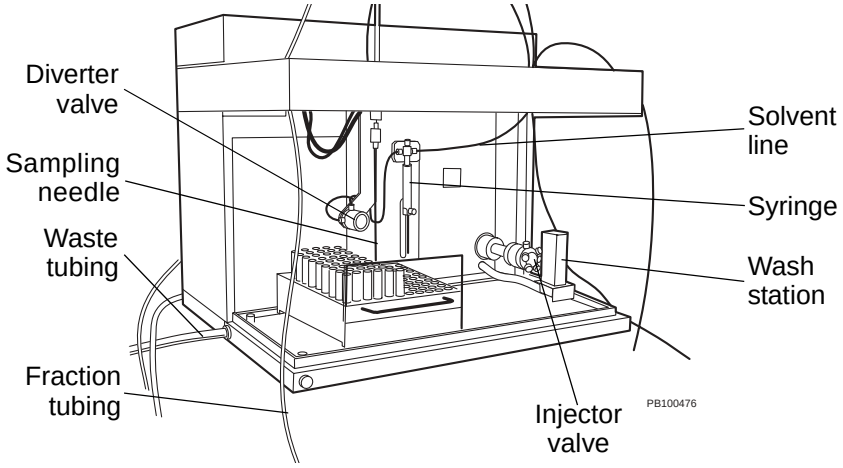


Figure 1-1 COBRA AFC 2000 Robotic Sample Handling Device

1.2 On Board Analysis™ Features

The AFC 2000 allows you to take advantage of the On Board Analysis™ features, specifically the Automated Analysis feature.

Automated Analysis

Automated Analysis integrates purification and analysis. This software feature automatically reinjects collected fractions when used in conjunction with the AFC 2000.

For more information on Automated Analysis, see Chapter 4, Using Automated Analysis.

1

Connecting the AFC 2000 Robotic Sample Handling Device

2

This chapter includes the following sections:

2.1 Overview

Parts list The COBRA AFC 2000 Robotic Sample Handling Device is provided with the following parts:

COBRA AFC 2000 Assembly Kit

- Feet, rubber, 3/4-inch (4)
- Washers, split (4)
- Screws, 12 mm (4)
- Plug, rubber, 1/4-inch (3)
- Plug, rubber, 3/16-inch (5)
- Shields, side (2)
- Sample loop, 100 µl
- Tubing, PEEK, 0.020 inch ID, 5 feet, orange
- Software, BioCAD, version 2.05
- *COBRA AFC 2000 Robotic Sample Handling Device User's Guide*

AFC 2000 Install Kit

- Power cord (1)
- Cable, communications, RS-232 (1)
- Shield, arm (1)
- Screw kit
- Wrench set, hex, metric
- Screwdriver, slot (1/8 x 2 inches)
- Racks (2), 13 mm test tube (holds 100 tubes)
- Test tubes, 13 x 100 mm (box of 250)
- Syringe, 500 µl
- Lubricant, syringe drive
- Rod, support (for coiled tubing)
- Fuse kit
- AFC 2000 Injector Valve Fitting Kit
- AFC 2000 Plumbing Kit

2.2 Assembling the AFC 2000

Before connecting the AFC 2000 to the BioCAD 700E Workstation, assemble the AFC 2000.

To assemble the AFC 2000:

- Route the waste tubing
- Attach feet
- Remove the protective packaging
- Insert the side plugs
- Attach the protective shields

Routing waste tubing

To route the waste tubing:

1. After removing the AFC 2000 from the box and plastic bag, set the device on its back.
2. Uncoil the waste tubing (1/2-inch OD, transparent, urethane) located on the underside of the instrument.
3. Route the waste tubing through the opening on the bottom-left side of the device (Figure 2-1).

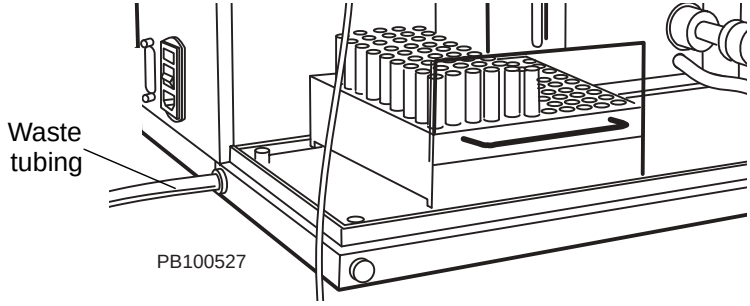


Figure 2-1 Routing Waste Tubing

CAUTION

After assembling and connecting the AFC 2000, place waste tubing in a waste container and place the waste container below the workstation. To prevent waste solution from backing up, make sure the tubing always slopes downward and does not become immersed in the waste solution.

Attaching feet

With the device still on its back, connect the four rubber feet to the underside of the AFC 2000 using the screws and split washers provided in the COBRA Assembly Kit (Figure 2-2).

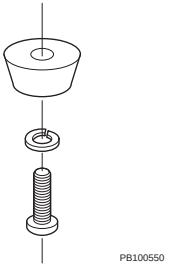


Figure 2-2 Attaching Feet

Removing protective packaging

To remove the protective transport packaging from the AFC 2000:

1. Set the AFC 2000 upright.
2. Remove the two hex screws on the back of the top cover.
3. Remove the top cover.
4. Remove the transport screw (red, hex) on the left side of the AFC 2000 (Figure 2-3).

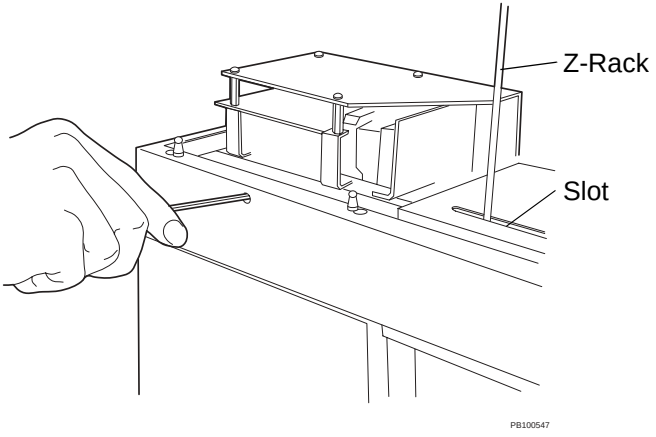


Figure 2-3 Removing the Transport Screw

5. Slide the autosampler arm to the right.
6. Remove the transport bracket from between the arm and the side of the AFC 2000 (Figure 2-4).

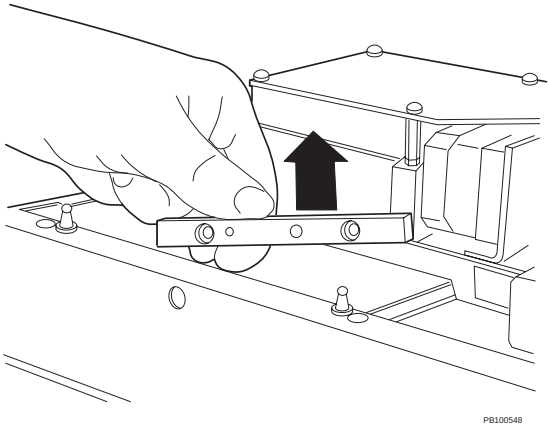


Figure 2-4 Removing the Transport Bracket

NOTE: *Save the transport screw and transport bracket for future shipping.*

7. Replace the top cover and hex screws.
8. Remove the foam piece from the Z-Rack slot (see Figure 2-3 on page 2-4).
9. Remove the tie wraps and slit tubing from the top and bottom of the Z-Rack.
10. Remove the tubing from the tip of the sampling needle.

Inserting side plugs

To insert the side plugs in the AFC 2000:

1. Insert the three 1/4-inch plugs (provided in the COBRA Assembly Kit). The plugs fit the three round holes on the sides of the AFC 2000 (two on the left, one on the right).
2. Insert the five 3/16-inch plugs (provided in the COBRA Assembly Kit). The plugs fit the five slotted holes on the sides of the AFC 2000 (two on the left, three on the right).

Attaching protective shields

To attach the protective shields in the AFC 2000:

1. Place the arm shield (provided in the AFC 2000 Install Kit) around the arm of the AFC 2000 (Figure 2-5).

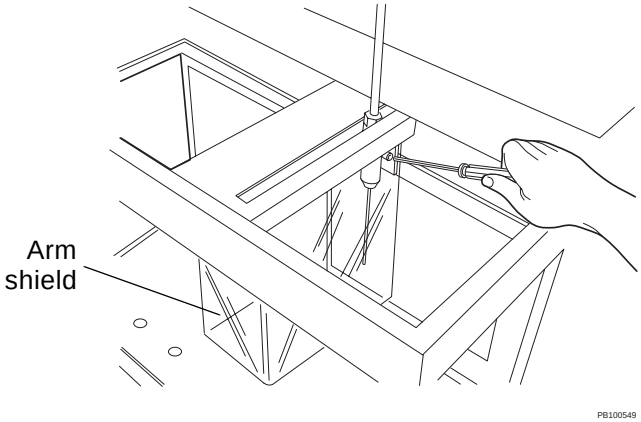


Figure 2-5 Attaching the Arm Shield

2. Attach the arm shield using the four hex screws (provided in the AFC 2000 Install Kit).
3. Attach the two side shields by sliding each into the top and bottom tracks on either side of the AFC 2000.

2.3 Connecting the AFC 2000

AFC 2000 power requirements

The AFC 2000 Robotic Sample Handling Device (see Figure 1-1 on page 1-3) requires a grounded outlet for its three-pronged plug. The power requirements are:

- **Power**—110–230 VAC, 47/63 Hz, 150 Watts
- **Fuses**—Two T3.15A 250V fuses

Other external devices

You can connect any two of the following instruments to a BioCAD 700E Workstation:

- SCOUT Column Selector
- Integrated Autosampler (AFC 2000 or AS 3500)
- External detector (UV/Vis or fluorescence)

NOTE: *You cannot connect both an AFC 2000 and an AS 3500 as integrated autosamplers.*

Connecting

To connect the AFC 2000 to the BioCAD 700E Workstation:

1. Place the AFC 2000 on top of the workstation. Make sure both the BioCAD 700E Workstation and the AFC 2000 are powered down.
2. Connect the power cord to the power receptacle on the left side of the AFC 2000 (Figure 2-6). Plug the power cord into an electrical outlet.

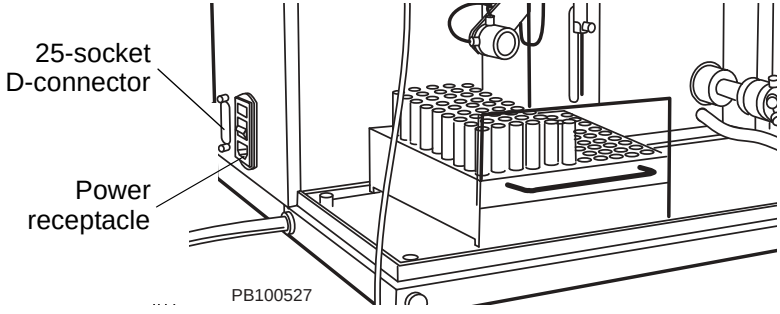


Figure 2-6 AFC 2000
Power Receptacle and 25-Socket D-Connector

3. Connect the cable supplied with the AFC 2000:
    - Connect the 25-pin D-connector to the 25-socket D-connector on the left side of the AFC 2000 (see Figure 2-6 on page 2-7).
    - Connect the 9-socket D-connector to a COM port on the left side panel of the BioCAD 700E Workstation (Figure 2-7). COM1 is recommended.

NOTE: *When you update the BioCAD Hardware Setup Program, select AFC 2000 as the autosampler and select the COM port you connected to above. See Section 2.6, Updating the BioCAD Hardware Setup Program.*

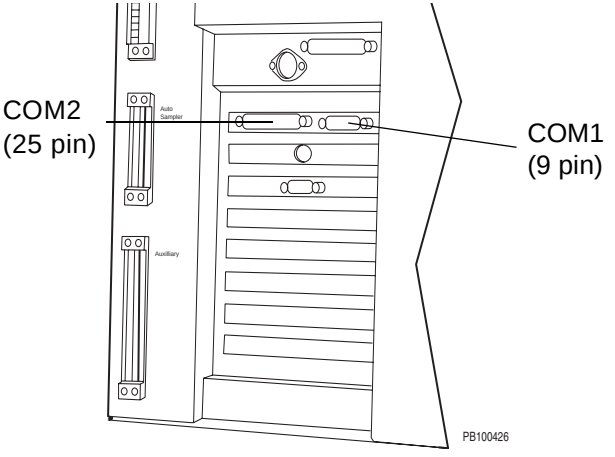


Figure 2-7 Connecting AFC 2000

NOTE: *The arrangement of boards in your computer may not exactly match Figure 2-7. To identify COM1, look for a 9-pin connector. To identify COM2, look for a 25-pin connector.*

4. Power up the AFC 2000 using the on/off switch on the bottom-left side panel. Power up the BioCAD 700E Workstation.

2.4 Plumbing the AFC 2000

To plumb the AFC 2000:

- Plumb the AFC 2000 injector valve
- Plumb the fraction tubing

2.4.1 Plumbing the AFC 2000 Injector Valve

Use the hex nut fittings to plumb the injector valve as shown in Figure 2-9 and Figure 2-10.

Preparing hex nut fittings

Use hex nut fittings to connect tubing to the 6-port injector valve.

To connect hex nut fittings to tubing:

1. Slip a compression fitting over the end of the tubing.
2. Slip a ferrule over the end of the tubing, with the tapered end pointing away from the compression fitting. Make sure at least 1/8-inch of tubing extends beyond the ferrule (Figure 2-8).

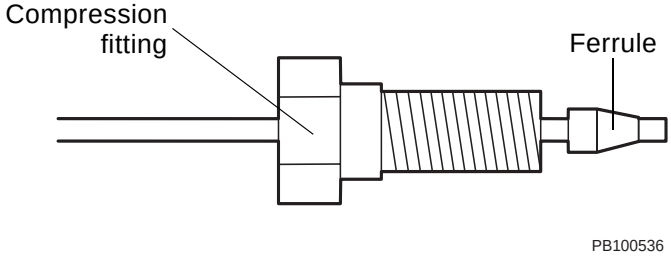


Figure 2-8 Connecting Hex Nut Fittings to Tubing

3. Insert tubing and attached ferrule into the 1/4-inch hexagonal union (provided in the AFC 2000 Injector Valve Fitting kit). Insert the tubing until the end is pressed against the inside of the union.
4. Use the 1/4-inch hex socket wrench (provided in the AFC 2000 Injector Valve Fitting Kit) to tighten the compression fitting until snug.

5. Loosen the fitting and remove the tubing from the union.

   The ferrule should be tight on the tubing so that it does not slide.

6. Insert the tubing in a port. Use the 1/4-inch hex socket wrench to tighten the compression fitting. Do not overtighten.

Plumbing the injector valve

The injector valve is located in the bottom-right corner of the device. The following plumbing configuration ensures that sample enters and exits the sample loop from the same end, minimizing sample dispersion in the loop.

Hint: *For easier access to the injector valve, you can remove the wash station by turning the set screw counterclockwise.*

NOTE: *After plumbing the AFC 2000 injector valve to the BioCAD 700E, you still have the option of injecting through the AFC 2000 injector valve or the BioCAD 700E injection valve.*

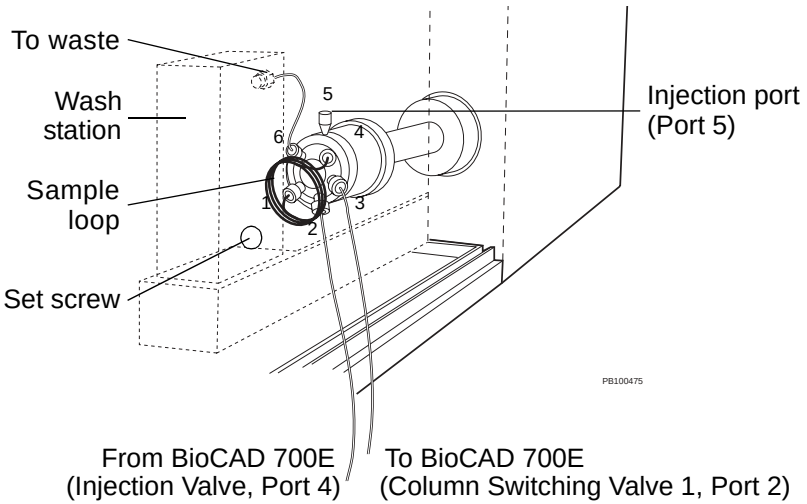


Figure 2-9 Plumbing Injector Valve AFC 2000 Robotic Sample Handling Device

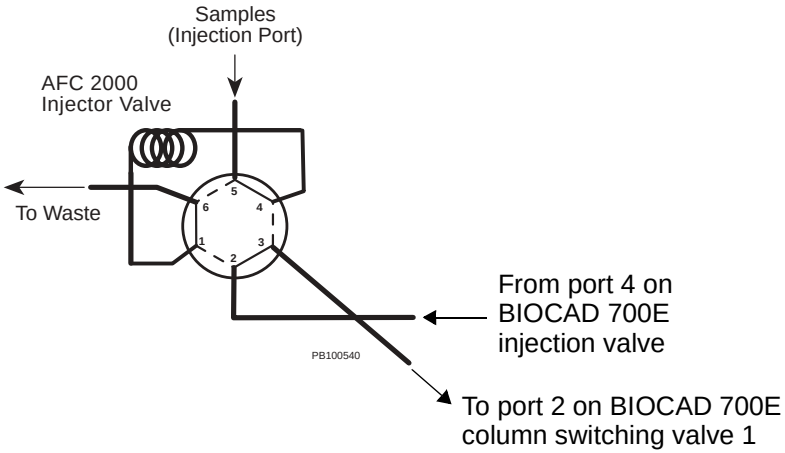


Figure 2-10 AFC 2000 Injector Valve Plumbing

To plumb the injector valve on the AFC 2000:

1. Plumb a 21-inch length of orange, 0.020-inch ID tubing from port 2 on the AFC 2000 injector valve to port 4 of the injection valve on the BioCAD 700E Workstation.
2. Plumb a 21-inch length of orange, 0.020-inch ID tubing from port 3 on the AFC 2000 injector valve to port 2 on Column Switching Valve 1 on the BioCAD 700E Workstation.
3. Plumb the sample loop between port 1 and port 4 on the injector valve. Attach the same size sample loop that you select when you configure the AFC 2000 (see Figure 3-11 on page 3-20).
4. Plumb from port 6 on the AFC 2000 injector valve to the top waste port on the wash station.
5. Increase the “Sample Injector to UV Detector” delay volume to account for the tubing you plumbed in step 1 and step 2. For more information, see “Delay volume” on page 3-25.

2.4.2 Plumbing the Fraction Tubing

Plumb the fraction tubing (1/16-inch OD Teflon®), which exits from the left side of the AFC 2000 (Figure 2-11), to the waste (bottom) port on the fraction/waste valve (Figure 2-12) on the BioCAD 700E Workstation.

NOTE: *If you change the length of the fraction tubing, change the delay volume for the tubing in the Configuration dialog box. For more information, see Section 3.2.1, Configuring as an Integrated Fraction Collector.*

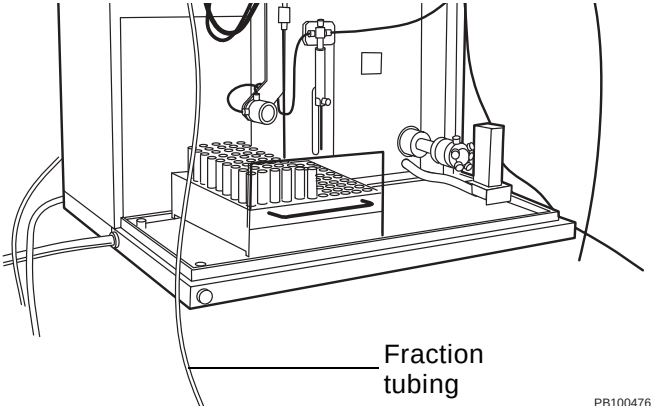


Figure 2-11 AFC 2000 Fraction Tubing

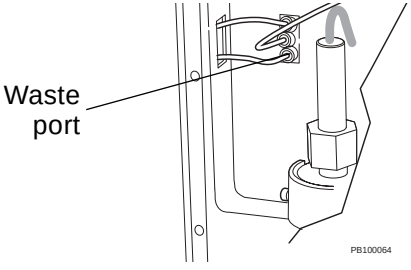


Figure 2-12 BioCAD 700E Workstation Fraction/Waste Valve

2.5 Installing the Software

The AFC 2000 requires BioCAD software version 2.05 or later. If your software version is earlier than 2.05, install the version 2.05 software provided in the COBRA Assembly Kit.

NOTE: *To determine the software version installed on your BioCAD 700E Workstation, select* ***About BioCAD*** *from the Window menu in the Control Panel.*

To install a new version of BioCAD software:

1. At the C:\> prompt, type **WIN** and press **Enter** to start Windows.
2. Insert BioCAD Disk #1 in the floppy drive at the top right of the BioCAD 700E Workstation.
3. Select **Run** from the File menu in the Program Manager window.

   The Run dialog box is displayed.
4. Type **A:\SETUP** in the Command Line and click **OK**.
5. Click **Continue** to bypass the first dialog box.

   A prompt asks you for the directory to install into.
6. Use the default of **C:\BIOCAD** and click **Continue**.
7. Change the disks as prompted.

NOTE: *If you delete the BioCAD software or replace your hard drive, contact Applied Biosystems Technical Support for assistance in re-installing the software.*

2.6 Updating the BioCAD Hardware Setup Program

Update the BioCAD Hardware Setup Program to recognize the AFC 2000 device.

Update procedure

To update the BioCAD Hardware Setup Program:

1. If you started the BioCAD software, exit by selecting **Exit BioCAD** from the File menu.
2. Double-click the **BioCAD Setup** icon in the BioCAD program window in the Windows Program Manager.

   The BioCAD Installation dialog box is displayed.

   NOTE: *If the BioCAD program window is not displayed, select* ***BioCAD*** *from the Window menu in the Windows Program Manager.*

3. Click **Continue**.

   The BioCAD Installation Options dialog box is displayed.
4. Select **Update Hardware Configuration** and click **Continue**.

   If you select Bring Back Old Configuration, the system reloads the previous configuration.
5. Follow the directions displayed on the screen.
6. When the BioCAD Hardware Setup Program prompts you to select the autosampler type, select **200–AFC 2000**. Click **Continue**.
7. Select the **COM port** (COM1 or COM2) to which you connected the AFC 2000. For more information, see “Connecting” on page 2-7. Click **Continue**.

   When the update is complete, a Setup Succeeded message is displayed.
8. Click **OK** to exit the BioCAD Hardware Setup Program.

2.7 Copying AFC 2000 Layout File

An AFC 2000 Layout File unique for your AFC 2000 is included with the device. This file is necessary to align the sampling needle. Copy the AFC 2000 Layout File to the hard drive of the BioCAD 700E Workstation.

To copy the AFC 2000 Layout File:

1. If you started the BioCAD software, exit by selecting **Exit BioCAD** from the File menu.
2. Remove the AFC 2000 Layout File disk that is attached to the bottom surface of the AFC 2000.
3. Insert the AFC 2000 Layout File disk in the floppy drive at the top right of the BioCAD 700E Workstation.
4. Double-click the **File Manager** icon in the Main group window of the Program Manager window.

   The File Manager window is displayed.
5. Copy the **AFC2000.LYT** file from the A directory (floppy disk drive) to the C:\BIOCAD directory.

   NOTE: *For more information on using File Manager to copy files, see the Microsoft® Windows® User's Guide.*
6. Select **Exit** from the File menu to exit File Manager.

2.8 Checking and Aligning the Sampling Needle

Checking

Check the alignment of the sampling needle to verify it is centered over the injection port. If the sampling needle is not centered, align the needle as described in “Aligning” on page 2-18.

To check the alignment of the sampling needle:

1. If you started the BioCAD software, exit by selecting **Exit BioCAD** from the File menu.
2. Select **Run** from the File menu in the Program Manager.

   The Run dialog box is displayed.
3. Type **SYSDIAG** in the Command Line text box. Click **OK**.

   The BioCAD System Level Diagnostics window is displayed.
4. Select **AFC 2000 Display** from the Windows menu.

   The AFC 2000 dialog box is displayed (Figure 2-13), and the AFC 2000 arm moves so that the sampling needle is in the home position (left, rear).

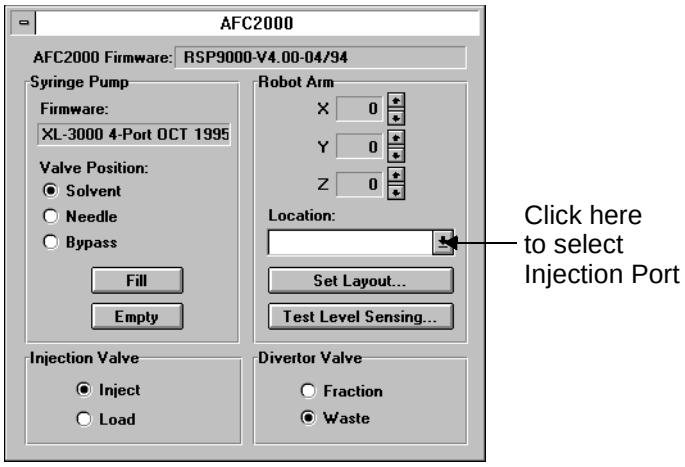


Figure 2-13 AFC 2000 Dialog Box

5. Select Injection Port from the drop-down list box.

   The AFC 2000 arm moves to the injection port position. The sampling needle should be centered in the injection port so that it creates a seal with the injection port sleeve. If the sampling needle is not aligned, see “Aligning” on page 2-18.

Aligning

Align the AFC 2000 sampling needle to center the needle over the injection port (see Figure 2-9 on page 2-10). Aligning the sampling needle sets the position of the sampling needle during injections.

To align the sampling needle:

1. If you did not check the sampling needle alignment, complete step 2 through step 4 on page 2-16.
2. Click the **Set Layout** button in the AFC 2000 dialog box (see Figure 2-13).

   The AFC 2000 Set Layout dialog box is displayed (Figure 2-14), and the AFC 2000 arm moves so that the sampling needle is over the injector valve.

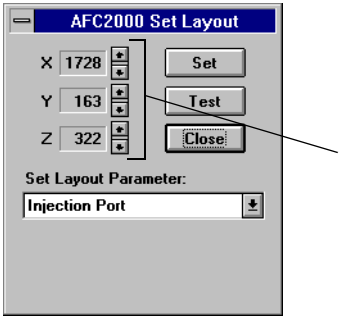


Figure 2-14 AFC 2000 Set Layout Dialog Box

3. Adjust the X (left, right) and Y (front, back) coordinates until the sampling needle is centered over the injection port.
4. Increase the Z (up, down) coordinate to lower the sampling needle to the injection port.
5. As the sampling needle nears the injection port, increase the Z coordinate one unit at a time until the needle starts to create a seal with the injection port sleeve.

NOTE: *To determine when the sampling needle starts to create a seal with the injection port, listen to the sound when you click the up arrow. The sound changes from a high-pitched click to a softer, muffled click.*

6. After the sound changes, increase the Z coordinate three units.

   The sampling needle is correctly aligned with the injection port.

7. Click **Set**. Click **Close**.

2.9 Starting the Software

There are two ways to start the BioCAD software:

- **From the C:\> prompt**—If you exit Windows, you can start the software from the C:\> prompt.

  Type **WIN BIOCAD** to start the software.

  NOTE: *To start Microsoft Windows without starting BioCAD software, type* ***WIN****.*

- **From the Windows Program Manager**—When you power up the system or exit the BioCAD software, double-click the **BioCAD** icon to start the software.

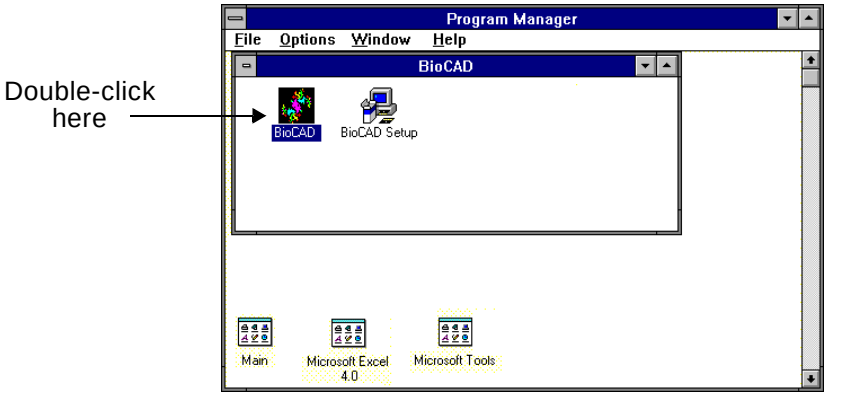


Figure 2-15 Program Manager with BioCAD Icon

NOTE: *If the BioCAD program window is not displayed, select it from the Window menu in the Windows Program Manager.*

The Control Panel is displayed when you start up the software.

Using the AFC 2000 Robotic Sample Handling Device

3

This chapter includes the following sections:

3.1 Preparing the AFC 2000 Robotic Sample Handling Device

Preparing as fraction collector

To prepare the AFC 2000 for use as an integrated fraction collector, you must load tubes, plates, and racks.
See Section 3.1.2, Loading Tubes, Plates, and Racks.

NOTE: *When using the AFC 2000 to collect fractions, you have the option of injecting through the AFC 2000 injector valve or the BioCAD 700E injection valve.*

Preparing as autosampler

To prepare the AFC 2000 for use as an integrated autosampler, you must perform the procedures in the following sections:

- Section 3.1.1, Preparing the Samples
- Section 3.1.2, Loading Tubes, Plates, and Racks
- Section 3.1.3, Attaching a Syringe and Sample Tubing
- Section 3.1.4, Preparing the Autosampler Solvent
- Section 3.1.5, Priming the Autosampler Solvent Line

3.1.1 Preparing the Samples

Before using the AFC 2000 as an integrated autosampler, prepare the samples as described below.

Filtering

Filter samples through a 0.22 or 0.45 micron filter before filling tubes or wells.

Filling tubes or wells

Fill tubes or wells with appropriate sample volumes.

CAUTION

To ensure smooth autosampler operation, use only tubes and microtiter plates provided by or recommended by Applied Biosystems. For ordering information, see Appendix B, Spare Parts.

Tube or Well	Maximum Volume	Dead Volume	
		*Liquid Level Sensing Disabled	*Liquid Level Sensing Enabled
2 ml Eppendorf® tube rack (100 conical tubes)	2.0 ml	20 µl	20 µl
13 mm test tube rack (100 tubes)	9 ml	100 µl	200 µl
16 mm test tube rack (90 tubes)	11 ml	100 µl	200 µl
50 ml plastic tube rack (25 conical tubes)	50 ml	120 µl	120 µl
96 well microtiter plate rack (2 plates per rack)	340 µl	50 µl	100 µl
***NOTE:** For information on Liquid Level Sensing, see page 3-59.*			

***NOTE:** The available volume for an injection is equal to the volume of sample in the tube minus the dead volume for the tube. The dead volume is the volume below which the software does not allow injections to be made.*

Sample cooling

You can cool samples by using either of the following racks:

- 16 x 100 mm chilled test tube rack (180 tubes)
- 13 x 100 mm chilled test tube rack (200 tubes)

For more information on loading and setting up these racks, see Section 3.1.2, Loading Tubes, Plates, and Racks.

3.1.2 Loading Tubes, Plates, and Racks

Before using the AFC 2000 as an integrated fraction collector or autosampler, load the tubes, plates, and racks in the device. Available tubes, plates, and racks include:

- 2 ml Eppendorf tube rack (100 conical tubes)
- 13 mm test tube rack (100 tubes)
- 16 mm test tube rack (90 tubes)
- 50 ml plastic tube rack (25 conical tubes)
- 16 mm chilled test tube rack (180 tubes)
- 13 mm chilled test tube rack (200 tubes)
- 96 well microtiter plate rack (2 plates per rack)

NOTE: *To ensure smooth AFC 2000 operation, use only racks provided by Applied Biosystems. Use only tubes and microtiter plates provided by or recommended by Applied Biosystems. For more information, see Table B-1 in Appendix B, Spare Parts.*

Loading tubes

Place tubes in racks. Each rack type holds a different number and size of tube. The first tube position is A:1 (left rack, left, rear position). The last tube position is B:# (right rack, right, rear position), where # represents the number of tubes the rack holds. The rack positions are ordered in a serpentine pattern with labels to the left of each position.

Loading microtiter plates

Place microtiter plates in racks as shown in Figure 3-1. Each rack holds two plates. In the software, the well positions are designated by rack letter, plate number, and well position. For example, B2:D3 refers to rack B, plate 2, well D3.

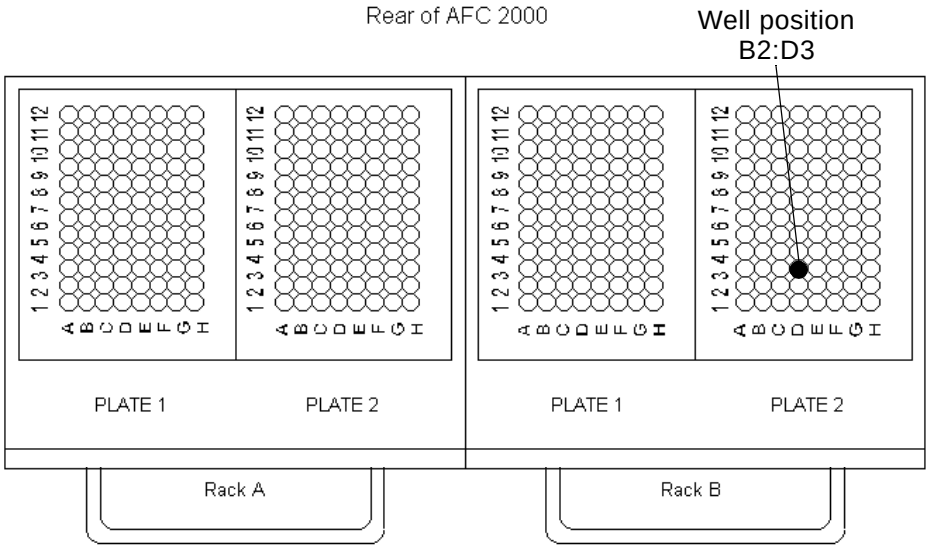


Figure 3-1 Microtiter Plate Racks

Fraction collector

If you are using the AFC 2000 as an integrated fraction collector, load an adequate number of empty tubes or plates for the fractions you will collect. Location of empty tubes in the rack is not important. However, you must enter Empty Fraction vial types in the Sample Table that correspond to the location of empty tubes or plate wells that you physically load in the racks.

Autosampler

If you are using the AFC 2000 as an integrated autosampler, load tubes or plates containing sample in the rack. Location of tubes in the rack is not important. However, you must enter Sample vial types in the Sample Table that correspond to the location of sample tubes or plate wells that you physically load in the racks.

Combined fraction collector/ autosampler

If you are using the AFC 2000 as a combined integrated fraction collector/autosampler, load empty tubes or plates (for fraction collection) and sample tubes or plates (for autosampling). Enter Empty Fraction or Sample vial types in the Sample Table that correspond to the location of tubes or plate wells that you physically load in the racks.

Loading racks

The AFC 2000 holds two racks. Rack A is on the left and Rack B is on the right. It is not necessary to install both racks before operating the AFC 2000.

CAUTION

Install the same type rack you configure for the AFC 2000. Installing a different rack can damage the sampling needle.

NOTE: *The 16 mm chilled test tube rack (180 tubes) and the 13 mm chilled test tube rack (200 tubes) occupy both rack positions.*

To load a rack:

1. Load tubes in the racks as described in “Loading tubes” or “Loading microtiter plates” on page 3-4.
2. Insert the racks so the holes on the underside of the rack fit over the locator pins on the bottom surface of the AFC 2000 (Figure 3-2).

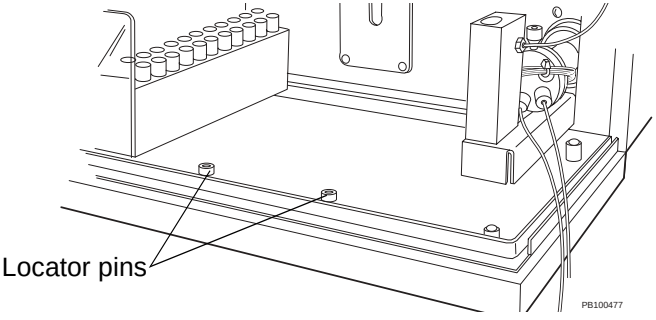


Figure 3-2 Installing Racks in AFC 2000

Connecting cooling lines

3. If you install the 16 mm chilled test tube rack (180 tubes) or 13 mm chilled test tube rack (200 tubes) to cool samples, attach tubing from a circulating water bath to the barbed fittings labeled Inlet and Outlet on the left side of the rack.

3.1.3 Attaching a Syringe and Sample Tubing

Before using the AFC 2000 as an integrated autosampler, attach a syringe to the syringe pump on the AFC 2000 (Figure 3-3). Use the syringe size you select when configuring the AFC 2000 (see Figure 3-8).

Attaching syringe

To attach a syringe:

1. Configure the AFC 2000 as an integrated autosampler. For more information, see Section 3.2.2, Configuring as an Integrated Autosampler.
2. Select **Change Syringe** from the Sample menu in the Sample Table.

   The syringe plunger retracts and the Set Syringe Size dialog box is displayed.
3. Unscrew and remove the screw located at the bottom front of the plunger.
4. Unscrew and remove the body of the existing syringe by turning it to the left.
5. Mount the new syringe on the plunger shaft and pull up on the syringe body until it reaches the fitting (Figure 3-3).

NOTE: *A 500 µl syringe is included.*

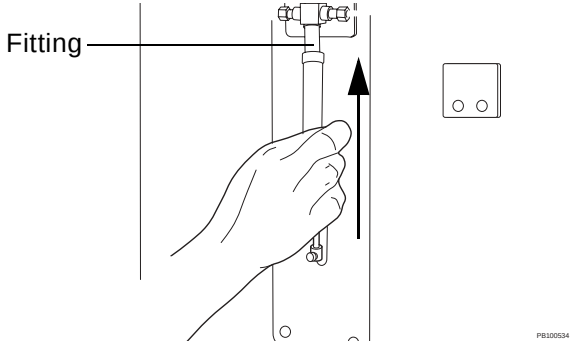


Figure 3-3 Attaching a Syringe to the AFC 2000

6. While pulling up gently, screw the syringe body into the fitting by turning it to the right.
7. Replace the screw at the bottom front of the plunger.
8. Select the **size** of the syringe you installed from the drop-down list box in the Set Syringe Size dialog box. Click **OK**.

   The syringe plunger returns to the home position.

NOTE: *The syringe size you select affects available flow rates. After changing the syringe size, you may need to reselect flow rates in the Configuration dialog box. For more information, see "Injection and Aspiration flow rates" on page 3-22.*

Attaching coiled tubing

If you attach a 2.5 ml or 5.0 ml syringe, you must also attach an extra length of coiled tubing between the syringe and the sample tubing to contain the larger sample volumes:

1. Install the rod and coiled tubing above the syringe by inserting the ends of the rod in the openings as shown in Figure 3-4. (The ends of the rod are identical).

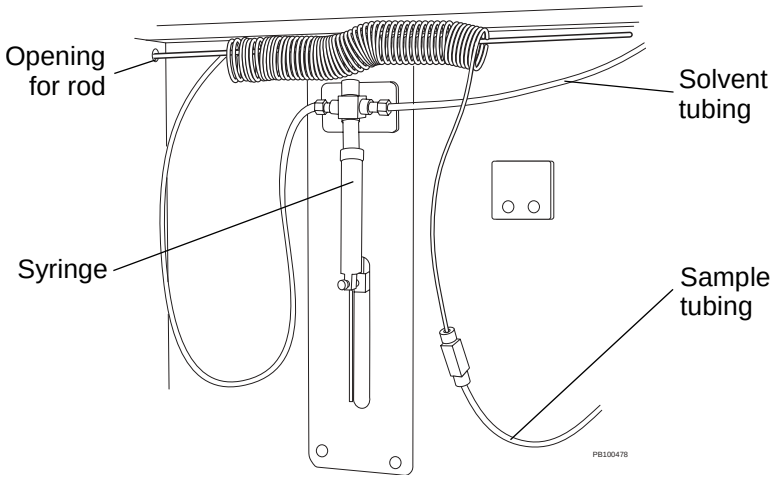


Figure 3-4 Installing Coiled Tubing

2. Disconnect the sample tubing from the left side of the syringe.
3. Connect the right end of the coiled tubing to the free end of the sample tubing.
4. Connect the left end of the coiled tubing to the left side of the syringe.
5. Prime the autosampler solvent line with at least 10,000 µl of autosampler solvent. For more information, see Section 3.1.5, Priming the Autosampler Solvent Line.

3.1.4 Preparing the Autosampler Solvent

Before using the AFC 2000 as an integrated autosampler, prepare an autosampler solvent for flushing, priming, chasing, and washing the device.

Selecting

For the autosampler solvent, use the reagent you configure as the autosampler solvent in the AFC 2000 Autosampler dialog box (see Figure 3-8).

NOTE: *Select a solvent that is appropriate for use with your samples. One to five percent isopropanol is appropriate for most applications.*

Filtering

Degas and filter all autosampler solvents through a 0.22 or 0.45 micron filter before use.

Locating

Insert the AFC 2000 solvent line (1/8-inch OD Teflon), which is attached to the right side of the syringe (Figure 3-5), in the solvent reservoir. Place the solvent reservoir on top of or to the right of the workstation.

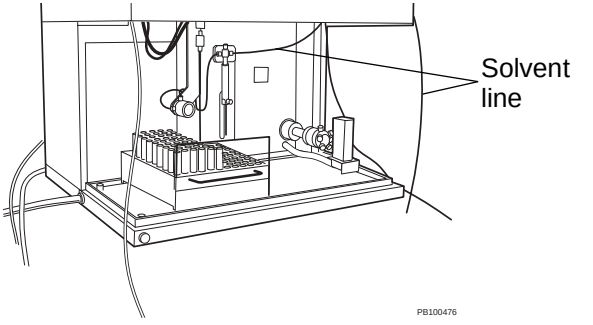


Figure 3-5 AFC 2000 Solvent Line

3.1.5 Priming the Autosampler Solvent Line

Before using the AFC 2000 as an integrated autosampler, prime the autosampler solvent line. Priming is necessary to fill the line and remove air bubbles.

To prime the autosampler solvent line:

1. Prepare the autosampler solvent as described in Section 3.1.4, Preparing the Autosampler Solvent.
2. Make sure you enter an appropriate prime volume when you configure the AFC 2000 as an integrated autosampler (see Figure 3-11). The prime volume should be large enough to fill the line and remove all air bubbles.

   NOTE: *If using a 500 µl syringe, enter a prime volume of at least 7,500 µl. If using a 2.5 or 5.0 ml syringe with an extra length of coiled sample tubing, enter a prime volume of at least 10,000 µl.*

3. Select **AFC 2000 Sample Table** from the Window menu to display the AFC 2000 Sample Table.

   NOTE: *If AFC 2000 Sample Table does not appear in the Window menu, verify that you specified an AFC 2000 for an autosampler in BioCAD Hardware Setup Program. For more information, see Section 2.6, Updating the BioCAD Hardware Setup Program.*

4. Select **Prime** from the Sample menu.

   The autosampler solvent line is primed with the prime volume you configured.

3.2 Configuring and Preparing the BioCAD Software for the AFC 2000

Before you begin

Before you configure the AFC 2000:

- Connect and power up the AFC 2000 using the on/off switch on the bottom-left side panel of the device
- Power up the BioCAD 700E Workstation and start the BioCAD software by double-clicking the BioCAD icon in the Windows Program Manager

Fraction collector

To configure and prepare the BioCAD software for use with the AFC 2000 as an integrated fraction collector:

- Configure the AFC 2000 as an integrated fraction collector as described in Section 3.2.1, Configuring as an Integrated Fraction Collector
- Enter Empty Fraction vial types in the Sample Table as described in Section 3.2.4, Entering Vials in Sample Table

Autosampler

To configure and prepare the BioCAD software for use with the AFC 2000 as an integrated autosampler:

- Configure the AFC 2000 as an integrated autosampler as described in Section 3.2.2, Configuring as an Integrated Autosampler
- Customize the Sample Library as described in Section 3.2.3, Customizing the Sample Library
- Enter Sample vial types in the Sample Table as described in Section 3.2.4, Entering Vials in Sample Table

Combined fraction collector/ autosampler

To configure and prepare the BioCAD software for use with the AFC 2000 as a combined fraction collector/autosampler, complete the tasks listed in “Fraction collector” and “Autosampler” above.

3.2.1 Configuring as an Integrated Fraction Collector

To configure the AFC 2000 as an integrated fraction collector:

1. Select **Edit Config** from the Config menu in the Control Panel to display the Configuration dialog box.
2. In the Collector section of the Configuration dialog box, (Figure 3-6) select **AFC 2000** from the list box.

NOTE: *If AFC 2000 is not available in the Collector section, verify that you specified an AFC 2000 for an autosampler in BioCAD Hardware Setup Program. For more information, see Section 2.6, Updating the BioCAD Hardware Setup Program.*

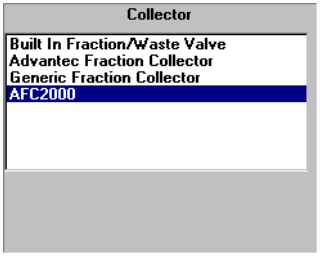


Figure 3-6 Configuring as an Integrated Fraction Collector

3. In the Autosampler/Fraction Collector section of the Configuration dialog box (Figure 3-7), click the **Configuration** button under Integrated AFC 2000.

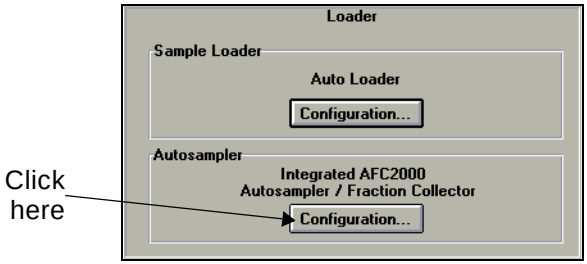


Figure 3-7 Configuring as an Integrated Fraction Collector

The AFC 2000 Autosampler/Fraction Collector dialog box is displayed (Figure 3-8).

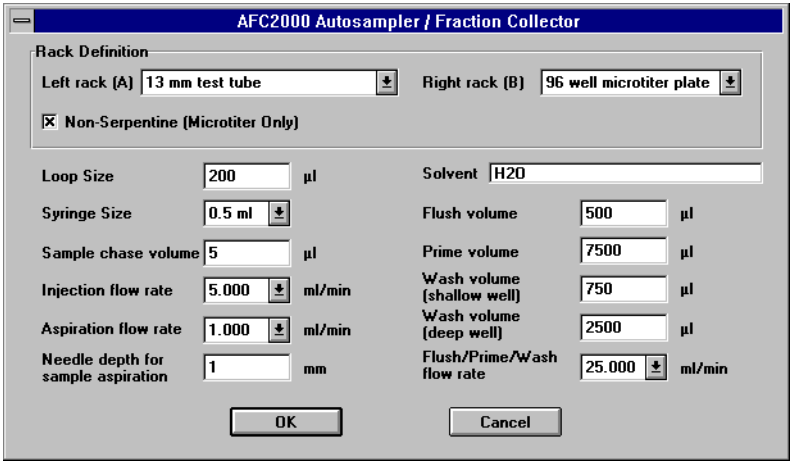


Figure 3-8 AFC 2000 Autosampler/Fraction Collector Dialog Box

Rack type

NOTE: *Configure the same type of rack that you install in the AFC 2000.*

4. From the Left Rack (A) drop-down list box, select the type of **rack** you inserted in the Left Rack (A) position of the AFC 2000. Choices are:

    - 2 ml Eppendorf tube rack (100 conical tubes)
    - 13 mm test tube rack (100 tubes)
    - 16 mm test tube rack (90 tubes)
    - 50 ml plastic tube rack (25 conical tubes)
    - 16 mm chilled test tube rack (180 tubes)
    - 13 mm chilled test tube rack (200 tubes)
    - 96 well microtiter plate rack (2 plates per rack)

    NOTE: *The 16 mm chilled test tube rack (180 tubes) and the 13 mm chilled test tube rack (200 tubes) occupy both rack positions.*

5. From the Right Rack (B) drop-down list box, select the type of **rack** you inserted in the Right Rack (B) position of the AFC 2000. Choices are:

    - 2 ml Eppendorf tube rack (100 conical tubes)
    - 13 mm test tube rack (100 tubes)
    - 16 mm test tube rack (90 tubes)
    - 50 ml plastic tube rack (25 conical tubes)
    - 96 well microtiter plate rack (2 plates per rack)

Filling pattern

6. If you select a microtiter plate rack, select or deselect the Non-Serpentine check box to control the filling pattern during fraction collection (Figure 3-9):

   - To collect fractions in the standard filling sequence, *select* the Non-Serpentine check box. Fractions are collected from front to back in each letter row. For example, well A1 to A12, B1 to B12, C1 to C12, and so on.
   - To minimize waste due to well switching time, *deselect* the Non-Serpentine check box. Fractions are collected from front to back in one row, and from back to front in the next row. For example, well A1 to A12, B12 to B1, C1 to C12, and so on.

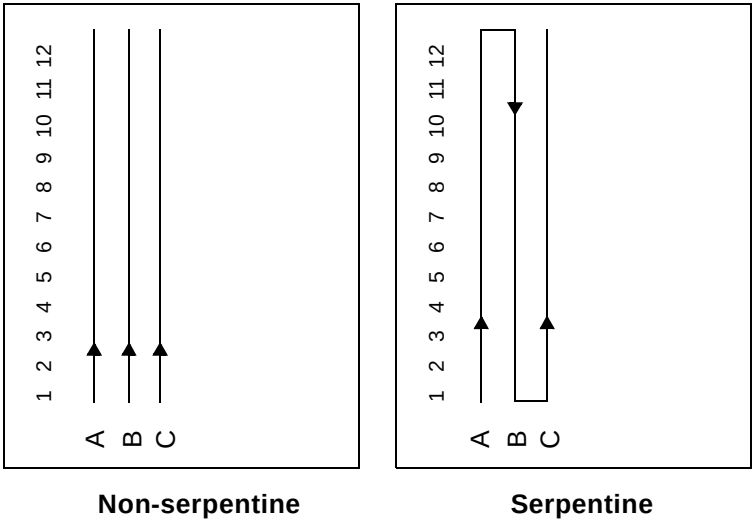


Figure 3-9 Microtiter Plate Filling Patterns

7. Click **OK** to return to the Configuration dialog box.

Delay volume

8. If you change the length of the fraction tubing between the fraction/waste valve and the AFC 2000, change the **delay volume** by clicking the Delay Volumes button in the Configuration dialog box. Type the correct delay volume in the Fraction Valve to Fraction Collector text box. For more information, see Configuring Columns and Plumbing, in the *BioCAD 700E Workstation User's Guide*.

   NOTE: *The default delay volume is 1.020 ml for the 60-inch, 0.03-inch ID fraction tubing.*

9. Click **OK** to save the configuration.

3.2.2 Configuring as an Integrated Autosampler

To configure the AFC 2000 as an integrated autosampler:

1. Select **Edit Config** from the Config menu in the Control Panel to display the Configuration dialog box.
2. In the Autosampler/Fraction Collector section of the Configuration dialog box (Figure 3-10), click the **Configuration** button under Integrated AFC 2000.

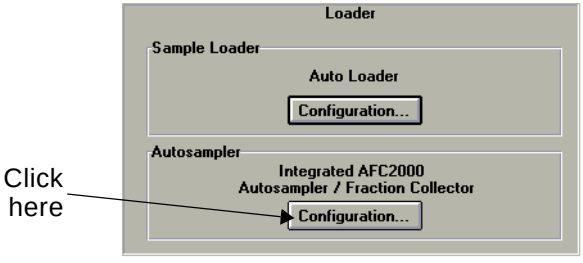


Figure 3-10 Configuring as an Integrated Autosampler

NOTE: *If Integrated AFC 2000 is not available in the Autosampler/Fraction Collector section of the Configuration dialog box, verify that you specified an AFC 2000 for an autosampler in BioCAD Hardware Setup Program. For more information, see Section 2.6, Updating the BioCAD Hardware Setup Program.*

The AFC 2000 Autosampler/Fraction Collector dialog box is displayed (Figure 3-11).

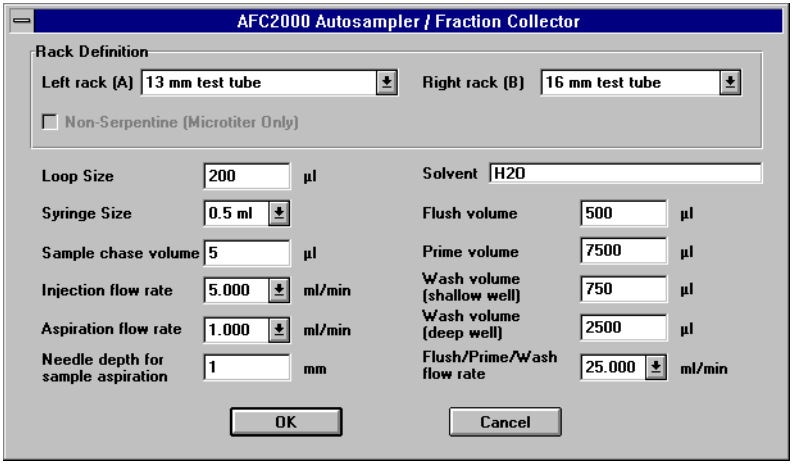


Figure 3-11 AFC 2000 Autosampler/Fraction Collector Dialog Box

3

Rack type

CAUTION

Configure the same type of rack that you install in the AFC 2000. Configuring a different rack can damage the sampling needle.

3. From the Left Rack (A) drop-down list box, select the type of **rack** you inserted in the Left Rack (A) position of the AFC 2000. Choices are:

    - 2 ml Eppendorf tube rack (100 conical tubes)
    - 13 mm test tube rack (100 tubes)
    - 16 mm test tube rack (90 tubes)
    - 50 ml plastic tube rack (25 conical tubes)
    - 16 mm chilled test tube rack (180 tubes)
    - 13 mm chilled test tube rack (200 tubes)
    - 96 well microtiter plate rack (2 plates per rack)

NOTE: *The 16 mm chilled test tube rack (180 tubes) and the 13 mm chilled test tube rack (200 tubes) occupy both rack positions.*

4. From the Right Rack (B) drop-down list box, select the type of **rack** you inserted in the Right Rack (B) position of the AFC 2000. Choices are:

    - 2 ml Eppendorf tube rack (100 conical tubes)
    - 13 mm test tube rack (100 tubes)
    - 16 mm test tube rack (90 tubes)
    - 50 ml plastic tube rack (25 conical tubes)
    - 96 well microtiter plate rack (2 plates per rack)

Loop size

5. In the Loop Size text box, type the **size** of the loop you attach to the injector valve of the AFC 2000. For more information, see Section 2.4.1, Plumbing the AFC 2000 Injector Valve.

Syringe size

6. From the Syringe Size drop-down list box, select the **size** of the syringe you connected to the syringe pump on the AFC 2000. For more information, see Section 3.1.3, Attaching a Syringe and Sample Tubing.

NOTE: *You can also change the AFC 2000 syringe and loop size from the Sample Table. The settings you make last are active. For more information, see Section 3.3, Controlling the AFC 2000 as an Integrated Autosampler.*

NOTE: *The syringe size you select affects available flow rates. After changing the syringe size, you may need to reselect flow rates in the Configuration dialog box. For more information, see "Injection and Aspiration flow rates" on page 3-22.*

Chase volume

7. Type the **Sample chase volume**.

    The chase volume is used to chase the sample from the sampling needle into the sample loop. The default is 5 µl.

Injection and Aspiration flow rates

NOTE: *Flow rates available depend on the syringe size you select. Select syringe size before selecting flow rate.*

8. Select an **Injection flow rate** and an **Aspiration flow rate** for injecting and aspirating sample. The default injection flow rate is 5.0 ml/min and the default aspiration flow rate is 1.0 ml/min.

CAUTION

Select an Aspiration flow rate slow enough to prevent cavitation of the syringe. Select an Injection flow rate slow enough to prevent overpressurization of the injection port. Appropriate flow rates depend on sample viscosity, sample volume, plumbing, and valve configuration.

Needle depth

9. Type the **Needle depth for sample aspiration**.

   The needle depth is the distance the needle tip is immersed in sample. We recommend using the default needle depth of 1 mm.

   The needle depth is used if you enable Liquid Level Sensing when adding an Autosampler Inject segment to a method. For more information, see “Adding an AFC 2000 Inject segment” on page 3-57.

Solvent

10. Select a reagent to use for the **autosampler solvent**:

    - Double-click on the **Autosampler solvent** field to display the Buffer/Solvent Library.
    - Select a reagent and click **OK**.

NOTE: *Select an autosampler solvent that is appropriate for use with your samples. One to five percent isopropanol is appropriate for most applications.*

NOTE: *The autosampler solvent is used for flushing, priming, chasing, and washing the AFC 2000.*

Flush volume

11. Type a **Flush volume** equal to or greater than three times your injection volume.

    The Flush volume is used to flush the needle and the sample line on the AFC 2000 after an injection. The Flush volume is used when you:

    - Flush the AFC 2000 when making an injection from the Sample Table. For more information, see Section 3.3, Controlling the AFC 2000 as an Integrated Autosampler.
    - Flush the AFC 2000 during an Autosampler Inject segment. For more information, see "Adding an AFC 2000 Inject segment" on page 3-57.

    NOTE: *The actual volume used for the flush is the flush volume you type OR a volume equal to three times the injection volume, whichever is larger.*

Prime volume

12. Type the **Prime volume**.

    The prime volume is used when you prime the solvent line on the AFC 2000. For more information, see Section 3.1.5, Priming the Autosampler Solvent Line.

    NOTE: *Type a prime volume of at least 7,500 µl to ensure complete priming of autosampler lines. Type a prime volume of at least 10,000 µl if you are using a 2.5 or 5 ml syringe with an extra length of coiled sample tubing. See "Attaching coiled tubing" on page 3-9.*

Wash volume

13. Type the **Wash volume (shallow well)** and **Wash volume (deep well)**.

    The *shallow* wash volume is used to wash the needle if you enable Liquid Level Sensing when adding an Autosampler Inject segment. The default shallow well wash volume is 750 µl.

    The *deep* wash volume is used to wash the needle if you do not enable Liquid Level Sensing when adding an Autosampler Inject segment. The default deep well wash volume is 2,500 µl.

    For more information, see “Adding an AFC 2000 Inject segment” on page 3-57.

Flow rate

NOTE: *The flow rates available depend on the syringe size you select. Select a syringe size before selecting flow rates.*

14. Select a **flow rate** for flushing, priming, and washing the AFC 2000. The default flow rate is 25 ml/min.

CAUTION

Select a flushing, priming, and washing flow rate slow enough to prevent cavitation of the syringe. Appropriate flow rates depend on reagent viscosity, reagent volume, plumbing, and valve configuration.

15. Click **OK** to return to the Configuration dialog box.

Delay volume

16. Increase the **delay volume** to account for the two pieces of 21-inch, 0.020-inch ID tubing (orange) you plumbed between the AFC 2000 and the BioCAD 700E injection and column switching valves:

    - Click the **Delay Volumes** button in the Configuration dialog box
    - Increase the delay volume in the “Sample Injector to UV Detector” text box

If you inject through the ...	Increase delay volume by ...
AFC 2000 Injector Valve	0.108 ml
BioCAD 700E Injection Valve	0.216 ml

    For more information, see Configuring Columns and Plumbing, in the *BioCAD 700E Workstation User’s Guide*.

17. Click **OK** to save the configuration.

3.2.3 Customizing the Sample Library

The Sample Library allows you to create, edit, and save samples for use with the AFC 2000. By customizing the Sample Library, you can quickly and easily retrieve information about specific samples when you create future configurations.

To customize the Sample Library:

1. Select **AFC 2000 Sample Table** from the Window menu in the Control Panel.

   The AFC 2000 Sample Table window is displayed (Figure 3-12).

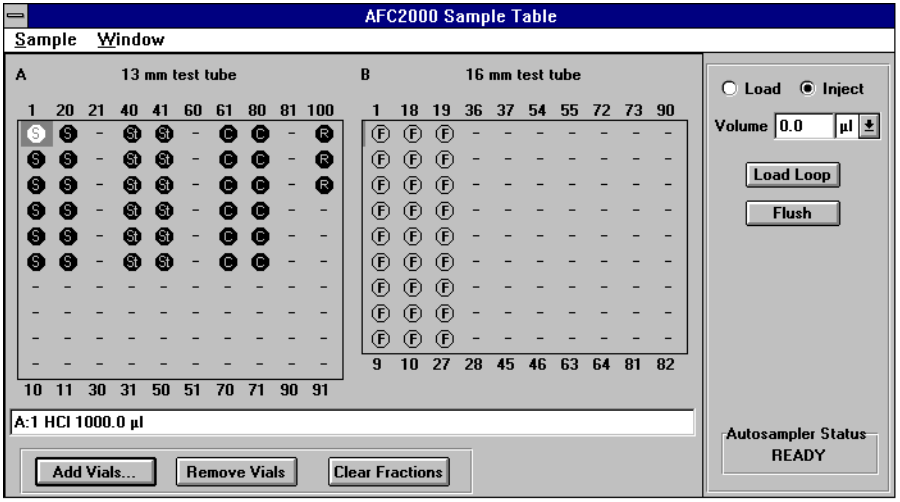


Figure 3-12 AFC 2000 Sample Table Window

NOTE: *If AFC 2000 Sample Table does not appear in the Window menu, verify that you specified an AFC 2000 for an autosampler in BioCAD Hardware Setup Program. For more information, see Section 2.6, Updating the BioCAD Hardware Setup Program.*

2. Double-click on any **vial position** in the grid.

   The AFC 2000 Vial Editor dialog box is displayed (Figure 3-13).

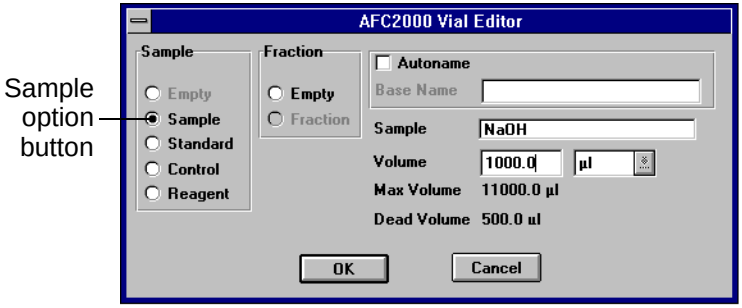


Figure 3-13 AFC 2000 Vial Editor Dialog Box

3. Click the **Sample** option button.

   The Sample Library dialog box is displayed (Figure 3-14).

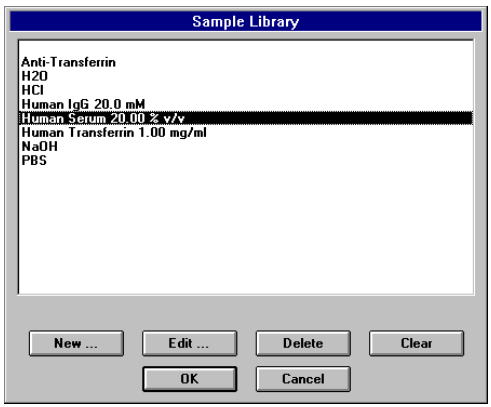


Figure 3-14 Sample Library Dialog Box

Creating or editing

4. To create a new sample, click the **New** button. To edit a sample, select the sample and click the **Edit** button.

   The Sample Editor dialog box is displayed (Figure 3-15).

NOTE: *All parameters that you type in the Sample Editor dialog box are used to identify the sample. Samples that have the same Short Name but different parameters are treated as different samples.*

In most locations in the software, all parameters are listed for a sample. However, the Short Name only is displayed in error codes and in Sample lists that appear in Inject Segment dialog boxes.

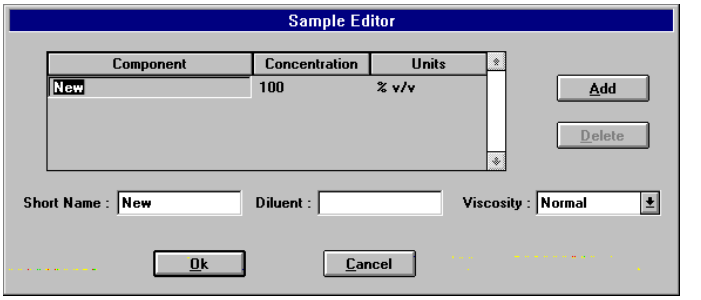


Figure 3-15 Sample Editor Dialog Box

5. Type or modify the following information:

Parameter	Description
Component	Type all component names in the sample. You must type at least one component name. To add additional components, click the **Add** button and type information on the line provided. Click the **Delete** button to remove the currently selected component.

Parameter	Description
Concentration and Units	Type concentrations and units for all components. The concentration of the first component defaults to 100%.
Short Name	Name displayed in error codes and in Sample lists (in Vial Editor, Inject Segment dialog box, and Analysis Template dialog box). ***NOTE:*** *Use unique Short Names for different samples. Samples that have the same Short Name but different parameters are treated as different samples.*
Diluent	Diluent that a sample contains. To add a diluent, type text in the field.
Viscosity	Not available at this time.

6. When you have added information for the desired parameters, click **OK**.

Deleting

7. To delete an entry from the Sample Library, select the **sample**, then click **Delete**.

CAUTION

Before clicking Delete, make sure you selected the correct sample. After you click Delete, you cannot retrieve the sample.

3.2.4 Entering Vials in Sample Table

This section describes:

- Overview
- Entering single vials or batches of vials
- Autonaming batch-entered vials
- Adding base name and suffix to Sample Library
- Removing vials
- Clearing fractions
- Saving Sample Table settings in a configuration

Overview

Enter vials in the Sample Table to correspond to the location of the tubes or microtiter plates you physically loaded in the racks in the AFC 2000. For more information, see Section 3.1.2, Loading Tubes, Plates, and Racks.

Fraction collector

If you are using the AFC 2000 as an integrated fraction collector, enter Empty Fraction vial types to correspond to the empty tubes or microtiter plates you loaded in the racks.

Autosampler

If you are using the AFC 2000 as an integrated autosampler, enter Sample, Standard, Control, or Reagent vial types to correspond to the tubes or microtiter plates containing sample that you loaded in the racks.

When you enter sample vials in the Sample Table window, the vials are automatically added to the current *system* configuration. When you run a method, the BIOCAD software compares the sample names and volumes requested by the method to the sample names and volumes entered in the Sample Table (system configuration).

If all sample names and volumes requested by the method are not listed in the Sample Table, a “Method’s configuration does not match...” error is displayed. Update your method or update the Sample Table and method configuration so that the sample names and volumes are consistent.

NOTE: *You cannot alter the Sample Table when a method is running. You can make an injection from the Sample Table when a method is running, if the AFC 2000 is not busy.*

Entering single vials or batches of vials

To enter single vials or batches of vials in the Sample Table:

1. Physically load sample tubes or microtiter plates in the AFC 2000 racks and load the racks. For more information, see Section 3.1.2, Loading Tubes, Plates, and Racks.

2. Select **AFC 2000 Sample Table** from the Window menu in the Control Panel.

   The AFC 2000 Sample Table window is displayed (Figure 3-16). The number, arrangement, and labeling of vials in the Sample Table reflect the type of rack you configured. For more information, see “Rack type” on page 3-20.

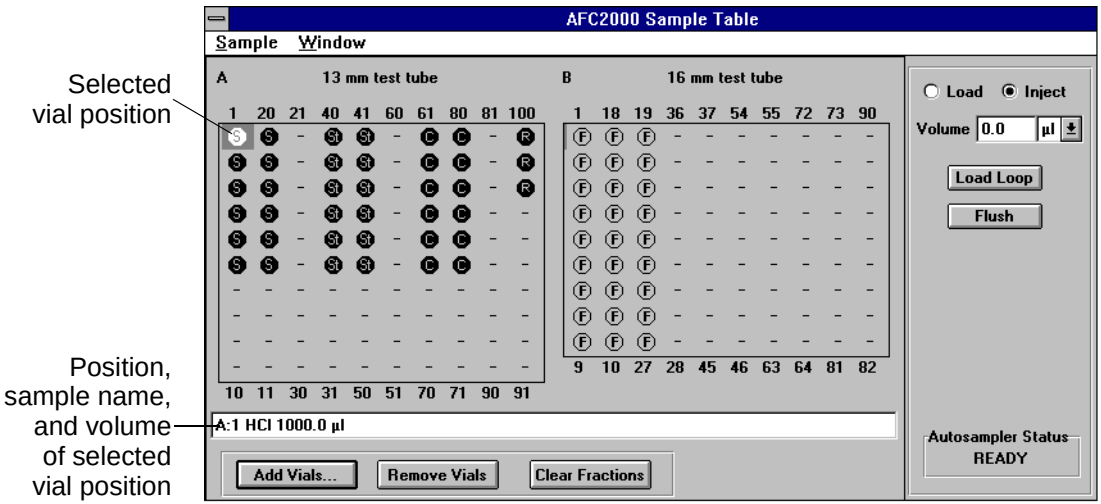


Figure 3-16 AFC 2000 Sample Table Window

3. To enter a single vial, double-click on a vial position in the Sample Table. To enter a batch of vials, click-drag over the vial positions and click **Add Vials**.

   The AFC 2000 Vial Editor dialog box is displayed (Figure 3-17).

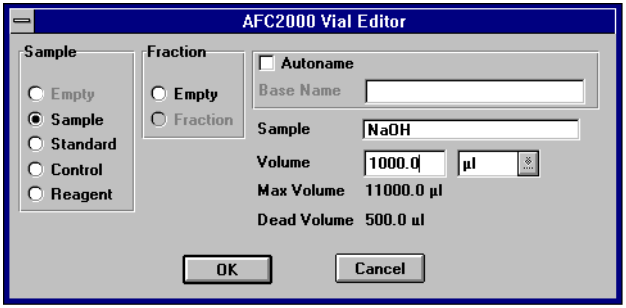


Figure 3-17 AFC 2000 Vial Editor Dialog Box

4. Select the **vial type**:

If you are using the AFC 2000 as ...	Select Vial Type ...	The software displays ...
Integrated autosampler	Sample, Standard, Control, or Reagent	The Sample Library. Select a **sample name** or add a new sample name and click **OK**. The Vial Editor dialog box is displayed again, with the name selected from the Sample Library displayed in the Sample text box. Continue with step 5. ***NOTE:*** *The sample vial is listed anywhere you select a sample vial in a method, for example, in an Inject Segment Setup dialog box.*
Integrated fraction collector	Empty (under Fraction section)	"Empty Fraction" in the Sample text box. The vial is entered. Continue with step 6. ***NOTE:*** *The vial will be used as the destination vial to collect fractions.*

NOTE: *If the vial has already been defined as Sample, Standard, Control, or Reagent, you can edit the sample by double-clicking the* ***Sample text box*** *to display the Sample Library. Select a* ***sample name*** *or add a new sample name and click* ***OK****. The Vial Editor dialog box is displayed again, with the name selected from the Sample Library displayed in the Sample text box.*
For information on adding names to the Sample Library, see Section 3.2.3, Customizing the Sample Library.

5. Type a **volume** for Sample, Standard, Control, or Reagent vial types. Allowable volumes are:

Tube or Well	Maximum Volume	Dead Volume	
		*Liquid Level Sensing Disabled	*Liquid Level Sensing Enabled
2 ml Eppendorf tube rack (100 conical tubes)	2.0 ml	20 µl	20 µl
13 mm test tube rack (100 tubes)	9 ml	100 µl	200 µl
16 mm test tube rack (90 tubes)	11 ml	100 µl	200 µl
50 ml plastic tube rack (25 conical tubes)	50 ml	120 µl	120 µl
96 well microtiter plate rack (2 plates per rack)	340 µl	50 µl	100 µl
***NOTE:** For information on Liquid Level Sensing, see page 3-59.*			

NOTE: *The available volume for an injection is equal to the volume of sample in the tube minus the dead volume for the tube. The dead volume is the volume below which no injection can be made.*

NOTE: *Type an accurate volume to avoid injecting from a tube with insufficient volume.*

NOTE: *As injections are made, the software recalculates the volume remaining in the tube. It accounts for volume used for injections and waste associated with making injections.*

6. Click **OK**.
7. Repeat step 3 through step 6 for all vials you want to enter in the Sample Table.

   The Sample Table window is displayed again, with all selected vial positions labeled with the designated vial type: Sample (S), Standard (St), Control (C), Reagent (R), or Empty Fraction (F). Click any vial position to display the position, sample name, and volume at the bottom of the Sample Table window (see Figure 3-16 on page 3-31).

NOTE: *Vial type (S, St, C, R) is for documentation purposes only.*

Test tube positions are designated by rack letter and vial number. For example, A:1 refers to rack A, position 1.

Well positions in microtiter plates are designated by rack letter, plate number, and well position. For example, B2:D3 refers to rack B, plate 2, well D3.

NOTE: *As you collect fractions, the vial positions in the Sample Table change from* Ⓕ *(Empty Fraction) to* ❶ *(Collected Fraction) to indicate that a fraction has been collected in the vial. Information about the collected fraction, including rack position and volume, is displayed at the bottom of the AFC 2000 Sample Table window.*

Hint: *Because you cannot alter the AFC 2000 Sample Table during a method run, you may want to enter "dummy" vials in the Sample Table to account for any vials you may need to add to the autosampler tray during a method run.*
Enter an appropriate volume and name (such as "unknown#") for the "dummy" vials. Be aware that you cannot update the sample name of the "dummy" vials in the final data files. This technique is not advisable for quality assurance procedures.

Autonaming batch-entered vials

You can use the Autoname feature when you batch-enter sample vials to specify a base name for the vials. The BIOCAD software adds a suffix (-1, -2, -3, and so on) to the base name. The sample name for each vial is the base name plus the suffix. You can also add the base name and suffix to the Sample Library.

Each time you use Autoname, the suffix resets to -1 (even if you use the same base name you used for a previous autoname).

To autoname batch-entered sample vials:

1. Physically load sample tubes in the AFC 2000 racks. For more information, see Section 3.1.2, Loading Tubes, Plates, and Racks.
2. Select **AFC 2000 Sample Table** from the Window menu in the Control Panel.

   The AFC 2000 Sample Table window is displayed (see Figure 3-16).
3. Select the sample vials to enter by click-dragging over the vial positions.

   The maximum number of sample vials you can add depends on the type of rack you installed and configured for the AFC 2000. Selected vials are highlighted.
4. Click the **Add Vials** button.

   The AFC 2000 Vial Editor dialog box is displayed (Figure 3-17).
5. Click the **Autoname** check box.

   The Base Name text box becomes active (Figure 3-18).

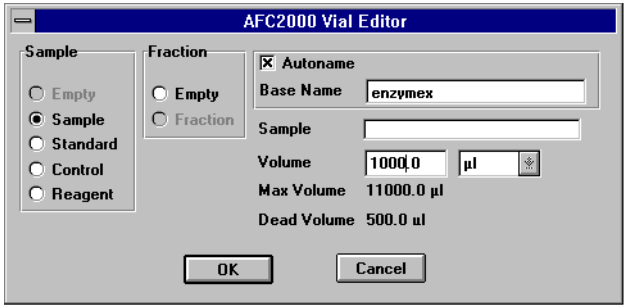


Figure 3-18 AFC 2000 Vial Editor with Base Name Text Box

6. Type a **Base Name** (up to 16 characters) for the vials.
7. Select the **vial type** (Sample, Standard, Control, or Reagent) from the Sample section of the dialog box. See step 4 on page 3-32 to determine vial type.
8. Type a **Volume**. See step 5 on page 3-33 to determine volume.
9. Click **OK**.

   The Sample Table window is displayed again, with all selected vial positions labeled with the designated vial type: Sample (S), Standard (St), Control (C), or Reagent (R), You can click any vial position to display the base name, suffix, and vial volume. The sample name for each vial is the base name plus the suffix.

NOTE: *Vial type (S, St, C, R) is for documentation purposes only.*

Adding base name and suffix to Sample Library

When you batch-enter vials using Autoname, you can also add the base name and suffix (sample name) to the Sample Library:

1. In the Sample Table window, double-click a vial position added using the Autoname feature.

   The Vial Editor dialog box is displayed.

2. Double-click the base name and suffix in the Sample text box.

   The Sample Library is displayed, with the base name and suffix added to the library list, with the following information:

   - **Short Name**—Base name and suffix (for example, SAMPLE-1)
   - **Sample Viscosity**—Normal
   - **Component Name**—Base name and suffix (for example, SAMPLE-1)
   - **Concentration/Units**—100%

   To change this information, see Section 3.2.3, Customizing the Sample Library.

Removing vials

To remove sample vials from the AFC 2000 Sample Table window:

1. Select the vial position to remove. Click-drag to select multiple vials.
2. Click the **Remove Vials** button.

   Selected vials are cleared from the AFC 2000 Sample Table window and from the current system configuration. Sample name information is *not* deleted from the Sample Library.

Clearing fractions

When the AFC 2000 collects fractions, the vial positions in the Sample Table change from Ⓕ (Empty Fraction) to ● (Collected Fraction). Information about the collected fraction, including rack position and volume, is displayed at the bottom of the Sample Table window.

To clear information associated with collected fractions from the Sample Table window:

1. Select the Collected Fraction (●) vial types you want to clear by click-dragging over the vial positions.
2. Click the **Clear Fractions** button.

   All selected Collected Fraction (●) vial types are cleared from the Sample Table window and replaced with Empty Fraction (Ⓕ) vial types.
3. Replace the tubes containing fractions with empty tubes.

Saving Sample Table settings in a configuration

You can save the settings in the Sample Table to a configuration file (.CFG) that you can use at a later time.

Select **Save Configuration As** from the File menu in the Control Panel. Type a file name for the configuration file. The Sample Table settings are saved to the configuration file.

If you want to use this configuration and Sample Table settings again, you can load the configuration by selecting **Open Configuration** from the Control Panel File menu.

3.3 Controlling the AFC 2000 as an Integrated Autosampler

WARNING

Keep hands out of the rack area when the AFC 2000 is in use.

AVERTISSEMENT

Veuillez garder les mains en dehors du bâti lorsque le AFC 2000 est utilisé.

Overview

If you are using the AFC 2000 as an integrated autosampler, you can control it from the Sample Table to make a single injection. You can also change the sample syringe and loop size from the Sample Table.

NOTE: *You cannot control the AFC 2000 from the Sample Table if you are running a method that uses the AFC 2000.*

Before you begin

Before you use the AFC 2000 to make an injection:

1. Connect the AFC 2000. See Section 2.3, Connecting the AFC 2000.
2. Prepare the AFC 2000. See Section 3.1, Preparing the AFC 2000 Robotic Sample Handling Device.
3. Configure and prepare the BioCAD software for use with the AFC 2000. See Section 3.2, Configuring and Preparing the BioCAD Software for the AFC 2000.
4. Purge the system as described in Purging the System, in the *BioCAD 700E Workstation User's Guide*.
5. Set the buffer/solvent blends for equilibrating as described in Controlling Buffers/Solvents and Blending, in the *BioCAD 700E Workstation User's Guide*.
6. Set the appropriate flow rate and start the pump as described in Controlling the Pump and Flow Rate, in the *BioCAD 700E Workstation User's Guide*.
7. Place the column inline to equilibrate as described in Controlling Columns, in the *BioCAD 700E Workstation User's Guide*.
8. Set the wavelength and zero the detector as described in Controlling Detectors, in the *BioCAD 700E Workstation User's Guide*.
9. Allow the pressure to stabilize by checking the pressure display in the Status window.

Making an injection

To make an injection from the Sample Table:

1. Select **AFC 2000 Sample Table** from the Window menu in the Control Panel.

NOTE: *If AFC 2000 Sample Table does not appear in the Window menu, make sure you selected an AFC 2000 in hardware configuration. For more information, see Section 2.6, Updating the BioCAD Hardware Setup Program.*

The AFC 2000 Sample Table window is displayed (Figure 3-19).

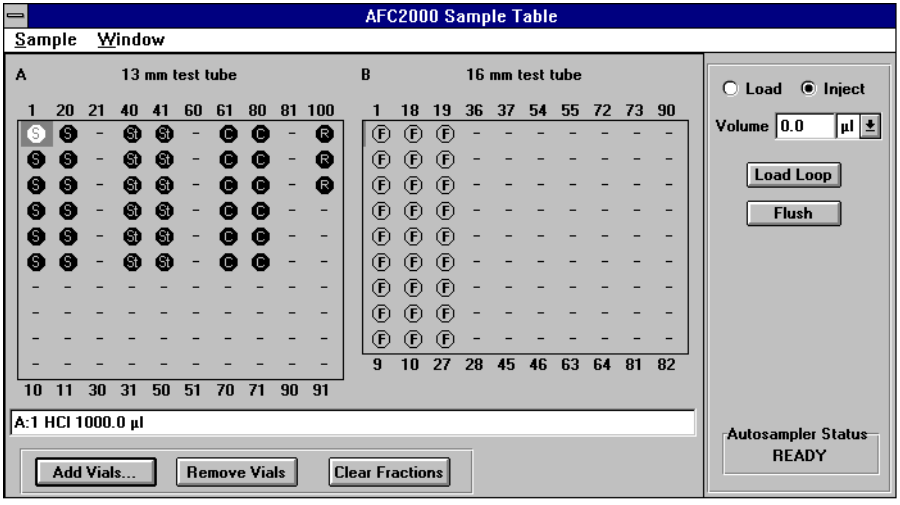


Figure 3-19 AFC 2000 Sample Table Window

2. Select a **sample vial** (S, St, C, or R) from the Sample Table by clicking it.
3. Type the **Volume** to inject in the Volume text box.

4. If desired, flush the autosampler needle:
    - Click the **Flush** button to display the Set Flush Amount dialog box.
    - Change the **flush volume**, if desired (the default volume is the volume you set in Configuration).
    - Click **OK** to flush the autosampler needle with the autosampler solvent set in Configuration.
5. Click the **Load Loop** button.

    The following occurs:
    - AFC 2000 injector valve rotates to Load position
    - AFC 2000 sample needle withdraws the specified volume from the selected tube
    - AFC 2000 sample needle loads the sample through the injection port into the sample loop
6. Click the **Inject** option button.

    The following occurs:
    - AFC 2000 injector valve rotates to Inject position
    - The sample is injected onto the column

NOTE: *Always inject the sample. You cannot load the loop again unless you inject the currently loaded sample.*

NOTE: *The autosampler injects from the vial you select in the Sample Table. If the sample volume in the vial is insufficient to make the injection, a warning is displayed. Click* ***OK*** *to reduce the injection volume or select a different vial. Click* ***Cancel*** *to cancel the injection.*

Changing sample loop or syringe size

You can change the AFC 2000 sample loop or syringe size from the Sample Table without changing Configuration:

1. Select **Change Loop Size** or **Change Syringe Size** from the Sample menu in the Sample Table.

   The Set Sample Loop Size or Set Syringe Size dialog box is displayed.

2. Type a new sample loop or syringe size and click **OK**.

3. Change the sample loop or syringe installed on the AFC 2000. For more information, see Section 3.1.3, Attaching a Syringe and Sample Tubing, and Section 2.4.1, Plumbing the AFC 2000 Injector Valve.

NOTE: *The syringe size you select affects available flow rates. After changing the syringe size, you may need to reselect flow rates in the Configuration dialog box. For more information, see* *"Injection and Aspiration flow rates" on page 3-22.*

3.4 Controlling the AFC 2000 as an Integrated Fraction Collector

WARNING

Keep hands out of the rack area when the AFC 2000 is in use.

AVERTISSEMENT

Veuillez garder les mains en dehors du bâti lorsque le AFC 2000 est utilisé.

Overview

If you are using the AFC 2000 as a fraction collector, you can control it from the Control Panel.

NOTE: *You cannot control the AFC 2000 from the Control Panel if you are running a method that uses the AFC 2000.*

Before you begin

Before you use the AFC 2000 to collect fractions:

- Connect the AFC 2000. See Section 2.3, Connecting the AFC 2000.
- Prepare the AFC 2000. See Section 3.1, Preparing the AFC 2000 Robotic Sample Handling Device.
- Configure and prepare the BioCAD software for use with the AFC 2000. See Section 3.2, Configuring and Preparing the BioCAD Software for the AFC 2000.

Collecting fractions

If you configure the AFC 2000 as a fraction collector, you can control it from the Collector section of the Control Panel:

NOTE: *If you change the length of the fraction tubing between the BioCAD 700E Workstation and the AFC 2000, change the* ***delay volume*** *by clicking the Delay Volumes button in the Configuration dialog box. Type the correct delay volume in the Fraction Valve to Fraction Collector text box. For more information, see Configuring Columns and Plumbing, in the BioCAD 700E Workstation User's Guide.*

---

1. Select **Fraction Collector Setup** from the Config menu in the Control Panel.

NOTE: *If Fraction Collector Setup does not appear, configure the AFC 2000 as a fraction collector. For more information, see Section 3.2.1, Configuring as an Integrated Fraction Collector.*

The Fraction Collector Setup dialog box appears (Figure 3-20).

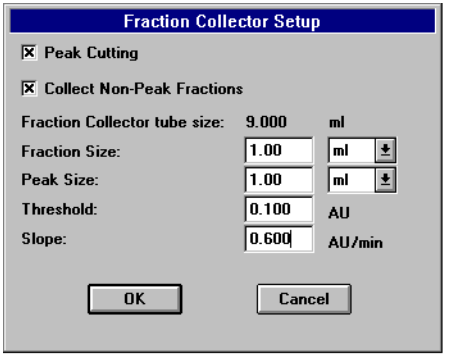


Figure 3-20 Fraction Collector Setup Dialog Box

2. Select and type fraction collector setup **parameters** in the dialog box. See “Setting fraction collection parameters” on page 3-54 for more information. Click **OK** to accept the settings and return to the Control Panel.

3. Click one of the following option buttons to divert the flow:

    - **Fraction**—Diverts flow to fraction collection tubes.
    - **Waste**—Diverts flow to waste.

Figure 3-21 Collector Section of Control Panel

4. Click the following buttons to control the fraction collection:

    - **Start/Stop**—Starts fraction collection according to the parameters you set in step 2. Stops fraction collection and diverts flow to waste if the AFC 2000 is collecting fractions.

    - **Advance**—Advances the diverter valve to the next available Empty Fraction tube in the rack sequence and collects flow.

    Hint: *Click the Advance button to control fraction collection if you do not set parameters in step 2.*

    - **Reset**—Moves the fraction collector diverter valve to the home position (left-rear tube) in the rack and diverts flow to waste.

Filling sequence

The filling sequence that the diverter valve follows depends on the rack you configured:

- **Any test tube rack**—The diverter valve starts at tube A:1 (left rear position in the rack) and moves in a serpentine pattern following the numbering on the rack.
- **Microtiter plate rack**—The diverter valve starts at well A1 (left front position in the plate) and moves in the pattern (serpentine or non-serpentine) you selected in the Configuration dialog box. For more information, see Section 3.2.1, Configuring as an Integrated Fraction Collector.

NOTE: *If you did not enter an Empty Fraction vial in the Sample Table in test tube position A:1 (rack) or well position A1 (microtiter plate), fraction collection does not start at this position. Fraction collection starts at the first available Empty Fraction vial position.*

As fractions are collected, the vial positions in the Sample Table change from Ⓕ (Empty Fractions) to ❶ (Collected Fractions) to indicate that a fraction has been collected in the vial.

The following information about the collected fraction is displayed at the bottom of the Sample Table window:

- Rack position
- Volume

This information about collected fractions is automatically added to the system configuration.

3.5 Creating and Running Methods With the AFC 2000

WARNING
Keep hands out of the rack area when the AFC 2000 is in use.

AVERTISSEMENT
Veuillez garder les mains en dehors du bâti lorsque le AFC 2000 est utilisé.

Fraction collector

You can collect fractions with the AFC 2000 by adding Fraction Collector events to a method.

Autosampler

You can make autosampler injections with the AFC 2000 by adding one of the following to a method:

- AFC 2000 Inject segment
- Step Segment containing Autosampler events

Combined fraction collector/ autosampler

You can collect fractions and inject samples with the AFC 2000 in the same method. However, do not set Fraction Collector events and Inject segments to occur at the same time within the method.

NOTE: *You can also use the AFC 2000 to automatically reinject collected fractions by using the Automated Analysis feature. For more information, see Chapter 4, Using Automated Analysis.*

Before you begin

Before you begin:

- Connect the AFC 2000. See Section 2.3, Connecting the AFC 2000.
- Prepare the AFC 2000. See Section 3.1, Preparing the AFC 2000 Robotic Sample Handling Device.
- Configure and prepare the BioCAD software for use with the AFC 2000. See Section 3.2, Configuring and Preparing the BioCAD Software for the AFC 2000.

The following sections describe:

- Adding Fraction Collector method events
- Adding an AFC 2000 Inject segment
- Adding Autosampler events

3.5.1 Adding Fraction Collector Events

You can control the AFC 2000 to collect fractions by adding Fraction Collector events to a method.

NOTE: *For basic information on creating and running methods, see Creating and Running Methods, in the BioCAD 700E Workstation User's Guide.*

The following table lists Fraction Collector events that you add to a method to control the AFC 2000 as an integrated fraction collector.

Fraction Collector Events (AFC 2000 Configured)	
Event	**Description**
Collect Fraction	Controls diverter valve on the AFC 2000 to divert flow to fraction collection tubes.
Divert to Waste	Controls diverter valve on AFC 2000 to divert flow to waste.
Start Fraction Collection	• Displays Fraction Collector Setup dialog box so you can set fraction collection parameters. See "Setting fraction collection parameters" on page 3-54. • Starts fraction collection in the next available empty fraction tube. ***NOTE:*** *Do not set a Start Fraction Collection event to occur at the same time an AFC 2000 Inject (AI) segment or an Inject Sample through AFC 2000 event is set to occur in a method.* ***NOTE:*** *If you add a Start Fraction Collection event to occur after an AFC 2000 injection that has flushing enabled, the Start Fraction Collection event is delayed until the flushing is complete.*

Fraction Collector Events (AFC 2000 Configured)	
Stop Fraction Collection	• Stops fraction collection. • Diverts flow to waste. • Advances diverter valve one tube. ***CAUTION:*** *After adding a Stop Fraction Collection event, allow time for the fraction tubing delay volume to elapse before ending the method. If you do not, later fractions will not be collected.* ***NOTE:*** *Add a Stop Fraction Collection event to use the AFC 2000 as an autosampler later in the method or after the method is complete.*
Advance to Next Tube	• Advances the diverter valve on the AFC 2000 to the next available Empty Fraction tube in the rack sequence. • Collects flow.
Reset Fraction Collector	• Moves the diverter valve on the AFC 2000 to the home position (left, rear) in the rack. • Diverts flow to waste. ***NOTE:*** *You cannot use the Reset event to pool fractions with an AFC 2000.*

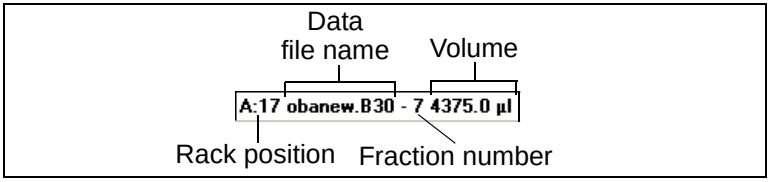
If you use the AFC 2000 as a fraction collector, the filling sequence that the diverter valve follows depends on the rack you configured:

- **Any test tube rack**—The diverter valve starts at tube A:1 (left rear position in the rack) and moves in a serpentine pattern following the numbering on the rack.
- **Microtiter plate rack**—The diverter valve starts at well A1 (left front position in the plate) and moves in the pattern (serpentine or non-serpentine) you selected in the Configuration dialog box. For more information, see Section 3.2.1, Configuring as an Integrated Fraction Collector.

NOTE: *If you did not enter an Empty Fraction vial in the Sample Table in test tube position A:1 (rack) or well position A1 (microtiter plate), fraction collection does not start at this position. Fraction collection starts at the first available Empty Fraction vial position.*

As fractions are collected, the vial positions in the Sample Table change from Ⓕ (Empty Fraction) to ❶ (Collected Fraction) to indicate that a fraction has been collected in the vial.

The following information about the collected fraction is displayed at the bottom of the Sample Table window:



This information about collected fractions is automatically added to the system configuration. The volume is adjusted when an injection is made from that tube.

NOTE: *Because the Information about collected fractions is added to the system configuration, but not the method configuration, if you rerun the method, you will get a Configuration Mismatch error.*

Setting fraction collection parameters

When you add a Start Fraction Collection event, the Fraction Collector Setup dialog box is displayed (Figure 3-22).

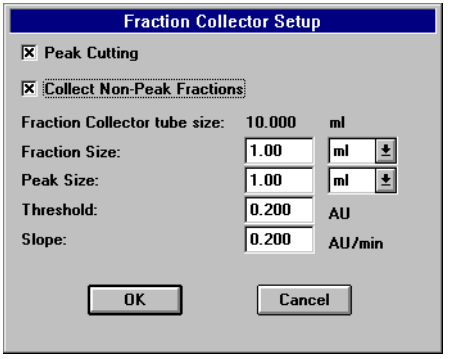


Figure 3-22 Fraction Collector Setup Dialog Box

The Fraction Collector Setup dialog box lets you specify:

- Only Collect Non-Peak Fractions
- Only Peak Cutting
- Both Peak Cutting and Collect Non-Peak Fractions

Only Collect Non-Peak Fractions

If you do not select Peak Cutting, Collect Non-Peak Fractions is enabled by default. The fraction collector collects fractions based on time or volume, whether or not peaks are detected (Figure 3-23).

Type a **Fraction Size**, in ml, min, or CV (column volume).

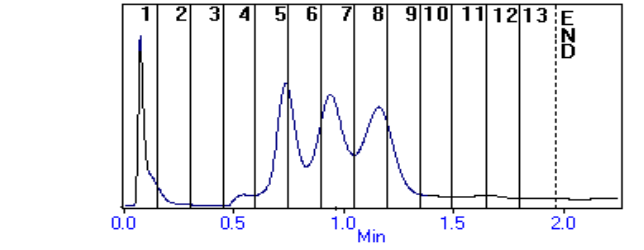


Figure 3-23 Collect Non-Peak Fractions

Only Peak Cutting

If you select only Peak Cutting, the fraction collector collects fractions only when a peak is detected, as specified by threshold and slope settings (Figure 3-24).

Type the following:

- **Peak Size**—Size of peak fractions to collect in min, ml, or CV (column volume). If actual peak exceeds the peak size setting, fractions are collected in multiple tubes.
- **Threshold**—Level in AU (absorbance units) that signals a peak.
- **Slope**—Rate of change in AU/minute that signals a peak.

NOTE: *Both threshold and slope values must be exceeded to trigger a peak. These values are based on UV/Vis Channel #1 in dual-wavelength systems. When peaks are not detected, the diverter valve on the fraction collector is set to waste.*

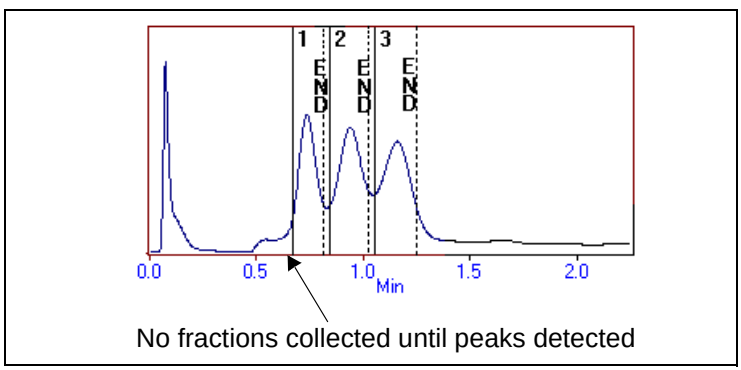


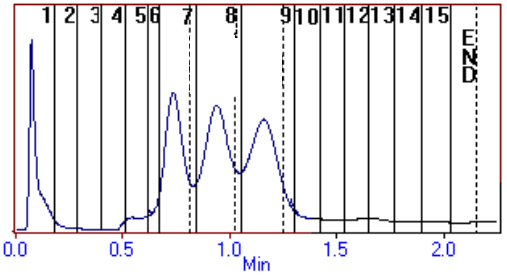
Figure 3-24 Peak Cutting

Both Peak Cutting and Collect Non-Peak Fractions

If you select both Peak Cutting and Collect Non-Peak Fractions, the fraction collector collects fractions based on time or volume until a peak is detected (Figure 3-25).

Type the following:

- **Fraction Size**—Size of non-peak fractions to collect in ml, min, or CV (column volume)
- **Peak Size**—Size of peak fractions to collect in ml, min, or CV (column volume)
- **Threshold**—Level in AU (absorbance units) that signals a peak
- **Slope**—Rate of change in AU/min that signals a peak



Uniform fractions collected, until peaks detected

Figure 3-25 Collect Non-Peak Fractions and Peak Cutting

3.5.2 Adding an AFC 2000 Inject Segment

You can make autosampler injections with the AFC 2000 by adding an AFC 2000 Inject (AI) segment to a method.

NOTE: *For basic information on creating and running methods, see Chapter 6, Creating and Running Methods, in the BioCAD 700E Workstation User's Guide.*

Adding an AFC 2000 Inject segment

To add an AFC 2000 Inject (AI) segment to a method:

1. Click on a **Load** block.
2. Click the Segment **AI** (AFC 2000 Inject) button.

NOTE: *If the Segment **AI** button is dimmed, enter sample or fraction vials in the Sample Table. For more information, see Section 3.2.4, Entering Vials in Sample Table.*

NOTE: *Do not set an AFC 2000 Inject (AI) segment to occur at the same time a Start Fraction Collection event is set to occur in a method.*
If you use the AFC 2000 to collect fractions earlier in the method, you must add a Stop Fraction Collection event to the method before adding an AFC 2000 Inject (AI) segment.

The AFC 2000 Inject Segment Setup dialog box is displayed (Figure 3-26).

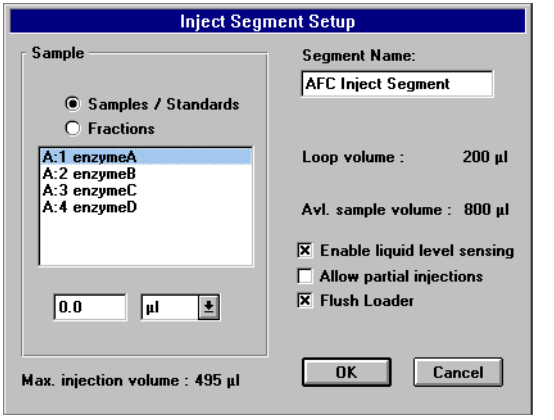


Figure 3-26 Inject Segment Setup Dialog Box (AFC 2000 Configured)

3. To inject a sample you prepared and loaded in the AFC 2000, click the **Samples/Standards** option button.

   The sample vials you entered in the Sample Table as Sample, Standard, Control, or Reagent are listed in the Sample list box.

4. To inject a fraction that the AFC 2000 collected, click the **Fractions** option button.

   The fraction vials you entered in the Sample Table are listed in the Sample list box.

5. Select a **sample** or **fraction** from the Sample list box.

   The dialog box displays the following for the selected sample vial:

   - **Maximum injection volume**—AFC 2000 syringe volume minus chase volume
   - **Available sample volume**—Volume of liquid in tube minus the dead volume for the tube

6. Type the **injection volume** in ml or µl.

   Valid range for an autosampler injection volume depends on the maximum injection volume and the available sample volume in the vial.

7. Type a **Segment Name** to identify the segment. The default segment name is AFC 2000 Inject Segment. The segment name appears on the Status window when a segment is running.

8. To flush the AFC 2000 sample needle with autosampler solvent after each injection, click the **Flush Loader** check box. (Flushing is recommended to minimize carryover.)

   Flush Loader flushes between the needle tip and the AFC 2000 injection syringe. The flush volume is three times the injection volume or the flush volume you set in the Configuration dialog box, whichever is larger.

   NOTE: *The AFC 2000 cannot collect fractions while it is flushing. If you want to collect fractions immediately after an injection, flush after fraction collection by adding a Flush AFC 2000 event.*

9. Enable or disable **Liquid Level Sensing**.

   NOTE: *You can enable Liquid Level Sensing with conductive samples such as buffers or samples containing tap water. Disable Liquid Level Sensing if you are using nonconductive samples such as distilled water, organics, or 100 percent acetonitrile.*

If you *enable* Liquid Level Sensing:

- When sampling, the needle enters the sample tube so that the needle tip is below the liquid surface. The depth that the needle is immersed in the liquid is equal to the needle depth you set in the Configuration dialog box. As the syringe withdraws liquid, the needle descends so that the tip of the needle is always the needle depth distance below the surface of the liquid. Sample waste is minimized.
- The needle is washed in the shallow well using the shallow well wash volume you set in the Configuration dialog box (see Figure 3-11). The needle is immersed to three times the depth it was immersed in the sample tube or the maximum depth of the well, whichever is smaller.

NOTE: *If no liquid is detected when Liquid Level Sensing is enabled, the AFC 2000 does not make an injection, but the method continues. An error is logged in the data file.*

If you *disable* Liquid Level Sensing:

- When sampling, the needle descends to the bottom of the tube.
- The needle is washed in the deep well using the deep well wash volume you set in the Configuration dialog box (see Figure 3-11). The needle is immersed to the maximum depth of the well.

10. Enable or disable **Partial Injections**.

    If you *enable* Partial Injections, and there is insufficient sample in a tube to make the requested injection, the AFC 2000 makes a partial injection and continues operation. The actual injection volume is documented in the system event log (SYSTEM.EVL) and the event log file (.EVL) that is stored with the data file.

    If you *disable* Partial Injections, and there is insufficient sample in a tube to make the requested injection, a warning is displayed before the method runs. The system:

    - Finds the tube with the same sample name and smallest adequate volume (if any) and asks if you want to inject from this vial. Click **OK** to inject from this vial, or click **Cancel** to cancel the method
    - If there is no tube with sufficient volume, the software asks if you want to reduce the injection volume. Click **OK** to reduce the injection volume, or click **Cancel** to cancel the method

    NOTE: *When running an Automated Analysis, if you disable Partial Injections and there is insufficient sample in a tube to make the requested injection, the analytical method does not run to analyze that sample, and an error is logged in the system event log file (SYSTEM.EVL).*

11. Click **OK**.

    An AFC 2000 Inject segment is added to the block.

3.5.3 Adding Autosampler Events

Adding Autosampler events

Instead of adding an AFC 2000 Inject (AI) segment you can also add Autosampler events to a Step segment in a method to make an injection using the AFC 2000. Adding individual method events gives you control over the order of events.

NOTE: *For basic information on creating and running methods, see Chapter 6, Creating and Running Methods, in the BioCAD 700E Workstation User's Guide.*

The following table lists Sample Load events that you add to a Step segment to control the AFC 2000 during injections:

Autosampler Events (AFC 2000 Configured)	
Event	**Description**
Set AFC 2000 Loop to Load Position	Specifies time at which to set injector valve to Load position.
Load Sample through AFC 2000	Specifies rack, sample, and volume to load, and time at which to load.

Continued

Autosampler Events (Continued) (AFC 2000 Configured)	
Inject Sample through AFC 2000	Specifies time at which to set injector valve to Inject position. ***NOTE:*** *To make an injection, include a Load Sample through AFC 2000 event before this event.* ***NOTE:*** *Do not set an Inject Sample through AFC 2000 event to occur at the same time a Start Fraction Collection event is set to occur in a method.* ***NOTE:*** *If you use the AFC 2000 to collect fractions earlier in the method, you must add a Stop Fraction Collection event to the method before adding an Inject Sample through AFC 2000 event.*
Flush AFC 2000	Specifies the time at which to flush and the volume of autosampler solvent to use. ***NOTE:*** *The AFC 2000 cannot collect fractions while it is flushing. If you want to collect fractions immediately after an injection, do not add a Flush AFC 2000 event until after fraction collection.*

Hint: *To collect fractions immediately after making an injection (for example, to analyze flow through from the column), add Autosampler events to a Step segment in the following order:*

Load Sample through AFC 2000
Inject Sample through AFC 2000
Start Fraction Collection
Stop Fraction Collection
Flush AFC 2000

Using Automated Analysis

4

This chapter includes the following sections:

4.1 Automated Analysis Overview

Overview On Board Analysis technology includes the Automated Analysis feature. Automated Analysis integrates purification and analysis. Automated Analysis uses the AFC 2000 Robotic Sample Handling Device to run two methods:

- **Preparative method**—Fractionates a crude sample
- **Analytical method**—Automatically runs repeatedly to inject, analyze, and quantitate the analyte in selected fractions

Setting up To set up an Automated Analysis, you:

- Create an analytical method
- Create a preparative method
- Create and save fraction analysis settings (including the analytical method) with the preparative method

Figure 4-1 illustrates the relationship between the preparative method, the analytical method, and the fraction analysis settings.

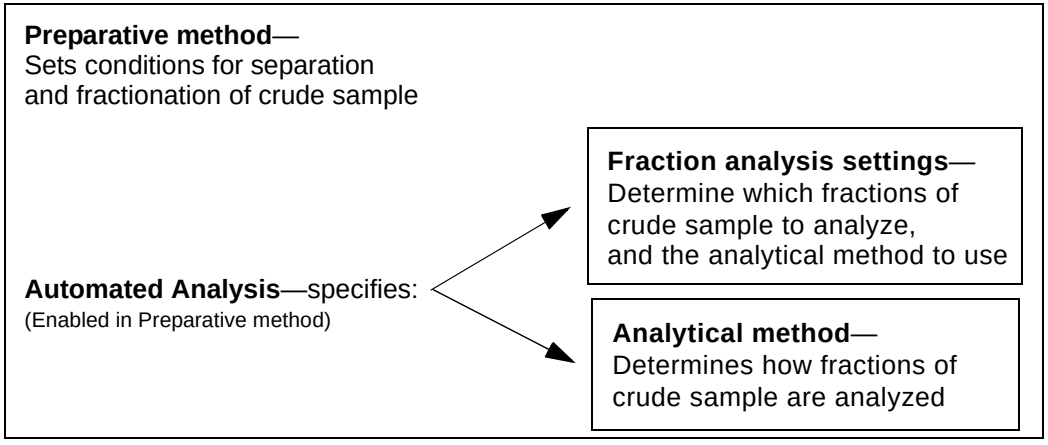


Figure 4-1 Preparative Method, Analytical Method, and Fraction Analysis Settings in Automated Analysis

Running To run an Automated Analysis, you run the preparative method. The analytical method runs also, as specified by the fraction analysis settings.

Figure 4-2 illustrates the process that occurs when you run an Automated Analysis.

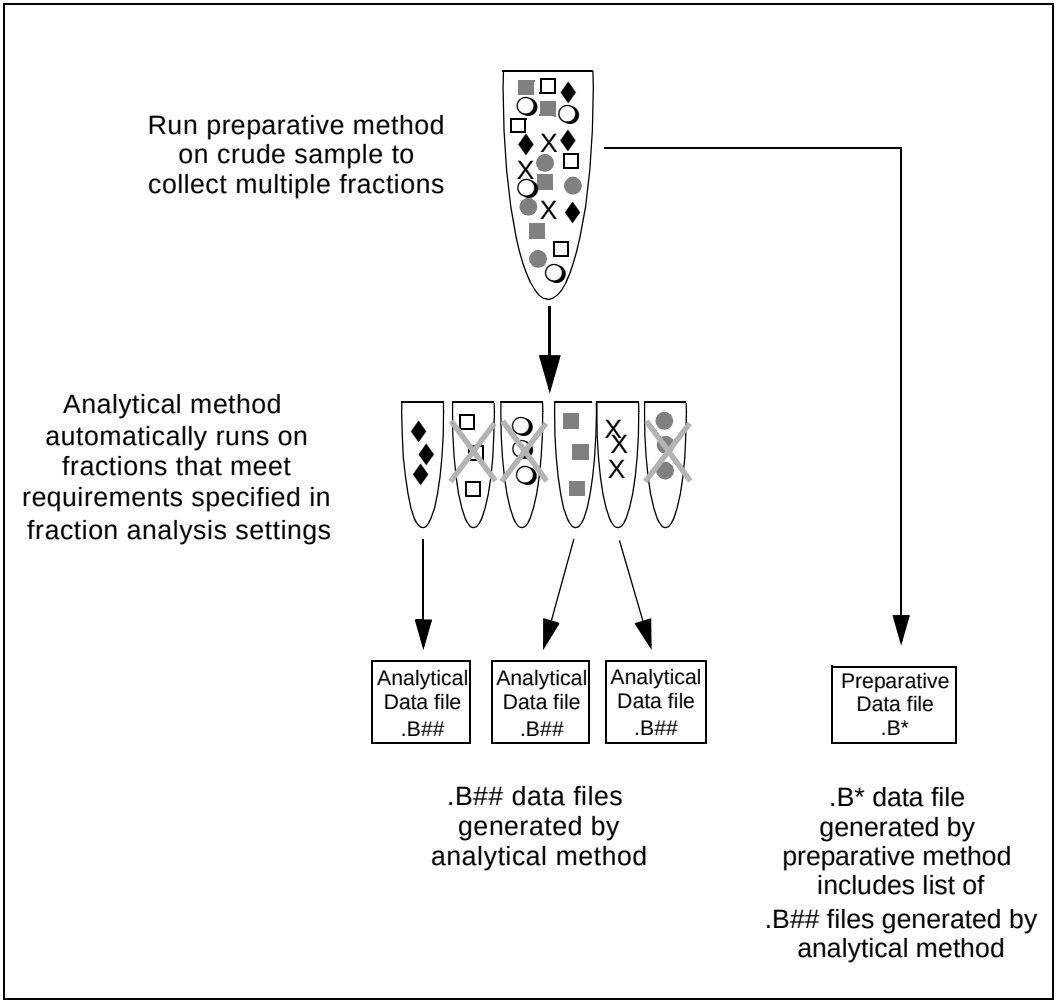


Figure 4-2 Automated Analysis Process

Templates and multi-methods

When you use Automated Analysis, you can also run a template or a multi-method. The template or multi-method makes repeated runs of the preparative method.

Figure 4-3 illustrates the process that occurs when you run a template or multi-method with Automated Analysis.

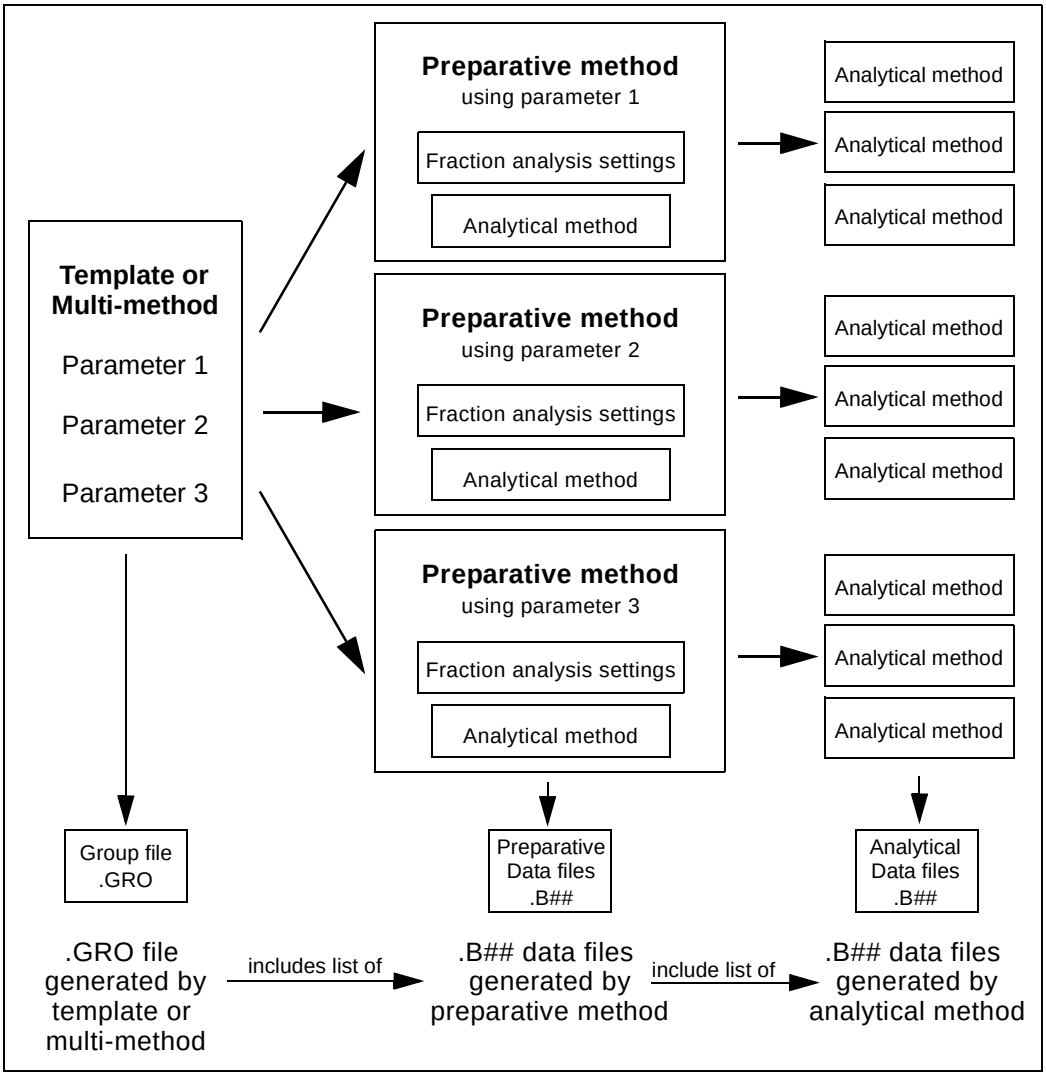


Figure 4-3 Templates or Multi-Methods with Automated Analysis

NOTE: *If you run a preparative method from a template, you have the option of disabling Automated Analysis by deselecting the Automated Fraction Analysis Settings check box in the template dialog box. Disabling Automated Analysis allows you to run the preparative method only, without using the Automated Analysis feature.*

The following sections describe:

- Automated Analysis requirements
- Setting up an Automated Analysis
- Running an Automated Analysis

4.2 Automated Analysis Requirements

Hardware requirements

Connect and prepare the AFC 2000 for use as a combined integrated fraction collector/autosampler. For detailed instructions, see Chapter 2, Connecting the AFC 2000 Robotic Sample Handling Device, and Section 3.1, Preparing the AFC 2000 Robotic Sample Handling Device.

Configuration requirements

Configure the AFC 2000 as a combined integrated fraction collector/autosampler. For detailed instructions, see Section 3.2, Configuring and Preparing the BioCAD Software for the AFC 2000.

Configure the Autoloader settings in the Configuration dialog box if your application requires you to inject your preparative sample through the injection valve on the BioCAD 700E Workstation.

Enter Sample and Empty Fraction vial types in the Sample Table. For more information, see Section 3.2.4, Entering Vials in Sample Table.

Method requirements

You must have both a preparative method and an analytical method to use Automated Analysis. Both methods must have the same configuration.

Methods must contain the following:

- **Preparative method**—
  - Fraction Collection Start event
  - Fraction Collection Stop event
  - Automated Fraction Analysis enabled
- **Analytical method**—AFC 2000 Inject (AI) segment

NOTE: *Examples of a typical preparative method (APREP.MET) and analytical method (ANALYT.MET) for use with the Automated Analysis feature are included in Appendix D, BioCAD Sample Methods.*

Buffer/solvent requirements

Select appropriate buffers/solvents for your preparative method and your analytical method.

Column requirements

Select a column appropriate for your preparative method and another column appropriate for your analytical method. The position you plumb each column will depend on your plumbing configuration. See “Plumbing requirements” below.

NOTE: *You can also plumb multiple columns for the preparative run and the analytical run by using the SCOUT Column Selector. See the SCOUT Column Selector User’s Guide for more information.*

Plumbing requirements

Your application determines your plumbing configuration. You can use a single-, tandem-, or three-column plumbing configuration with Automated Analysis. You can also use the SCOUT Column Selector to plumb additional columns for multi-dimensional preparations or analyses.

Multiple preparative columns

If you plumb multiple preparative columns, you must do one of the following:

- Run a separate preparative method for each column, each with Automated Analysis enabled.
- Run a Column Switching template on the preparative method. For more information on the Column Switching template, refer to the *SCOUT Column Selector User’s Guide*.

Multiple analytical columns

When you use Automated Analysis, each preparative method can contain and run only one analytical method with one analytical column.

If you plumb multiple analytical columns, you can analyze the collected fractions using additional analytical methods and columns. Run an Analysis template or Column Switching template on each additional analytical method.

For more information on the Analysis template, see Analysis Template (Integrated Autosampler), in the *BioCAD 700E Workstation User’s Guide*. For more information on the Column Switching template, see the *SCOUT Column Selector User’s Guide*.

Example Figure 4-4 illustrates a typical tandem-column plumbing configuration for use with Automated Analysis. Use this plumbing configuration with the Automated Analysis sample methods (APREP.MET and ANALYT.MET) listed in Appendix D, BioCAD Sample Methods.

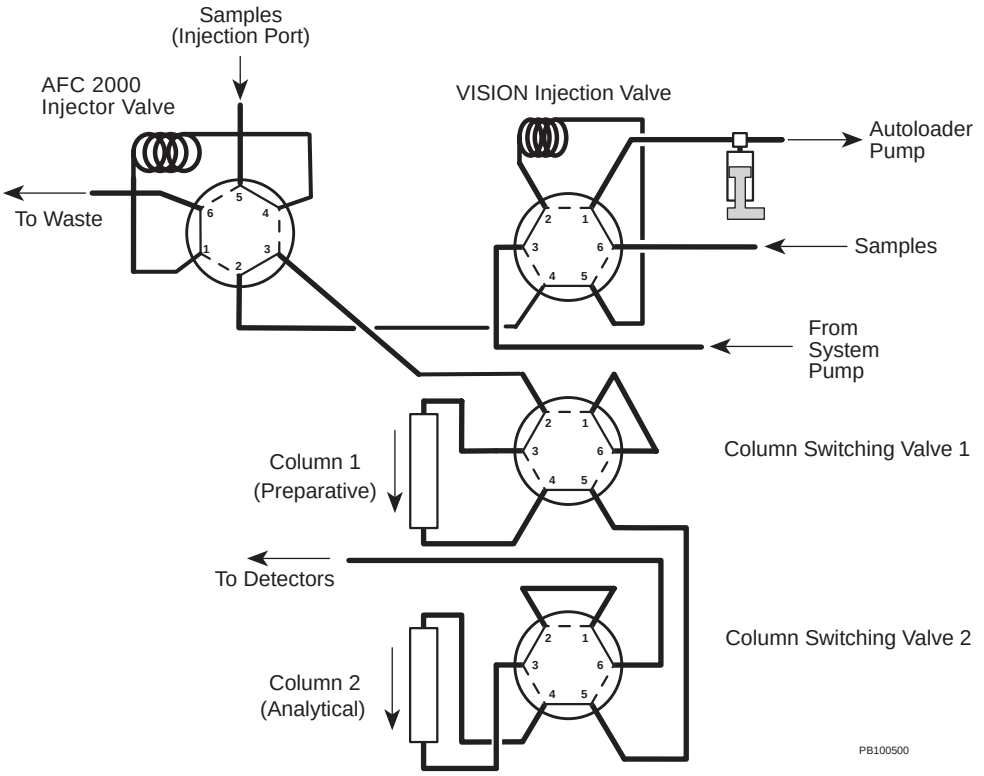


Figure 4-4 Typical Plumbing Configuration for Automated Analysis

4.3 Setting Up an Automated Analysis

To set up an Automated Analysis:

1. Connect, prepare, and configure the AFC 2000 as a combined integrated fraction collector/autosampler.

   For more information, see Chapter 2, Connecting the AFC 2000 Robotic Sample Handling Device, Section 3.1, Preparing the AFC 2000 Robotic Sample Handling Device, and Section 3.2, Configuring and Preparing the BioCAD Software for the AFC 2000.

2. Enter an adequate number of Empty Fraction vial types in the Sample Table for the fractions you will collect.

   For more information see Section 3.2.4, Entering Vials in Sample Table.

3. Create and save an analytical method.

   An example of a typical analytical method (ANALYT.MET) for use with the Automated Analysis feature is included in Appendix D, BioCAD Sample Methods.

4. Open or create a preparative method.

   An example of a typical preparative method (APREP.MET) for use with the Automated Analysis feature is included in Appendix D, BioCAD Sample Methods.

5. With the preparative method open, select **Automated Fraction Analysis** from the On Board Analysis menu in the Method Editor.

   A check mark appears next to the Automated Fraction Analysis command to indicate it is enabled.

6. Select **Fraction Analysis Settings** from the On Board Analysis menu in the Method Editor.

   The Automated Fraction Analysis Settings dialog box is displayed (Figure 4-5).

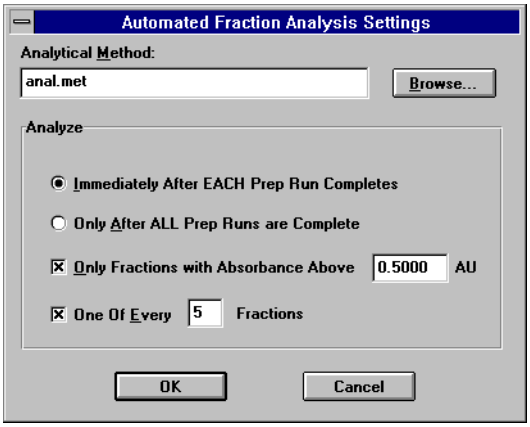


Figure 4-5 Automated Fraction Analysis Settings Dialog Box

7. Type the **file name** of the analytical method you created in step 3 in the Analytical Method text box or click **Browse** to select the analytical method from the Open dialog box.

NOTE: *If you do not select Fraction Analysis Settings and enter information in the dialog box, your preparative method will run using the default Automated Analysis settings. You will be prompted for a analytical method file name before the preparative method runs.*

---

8. If you will use a template or multi-method to run the preparative method multiple times, select one of the following option buttons:

   - **Immediately After EACH Prep Run Completes**—Runs the analytical method after each preparative method run is complete. This option button is selected by default.

Hint*: Select the above option if your samples are labile or if you need results quickly.*

   - **Only After ALL Prep Runs Are Complete**—Runs the analytical method after all preparative method runs are complete.

9. To set a minimum absorbance (AU) threshold for the fractions you analyze, click the **Only Fractions with Absorbance Above** check box and type an **absorbance value** (AU). The value must be less than or equal to 3.0. This check box is disabled by default.

10. To analyze only a subset of collected fractions, click the **One of Every __ Fractions** check box and type a **number**. The number must be between 2 and 99. This check box is disabled by default.

NOTE: *If you select both of the above check boxes, fractions that meet either criteria will be analyzed.*

NOTE: *The analytical method can analyze a maximum of 100 fractions. If your preparative method produces more than 100 fractions, you can analyze the remainder by opening the analytical method and running an Analysis template. For more information, see Analysis Template (Integrated Autosampler), in the BioCAD 700E Workstation User's Guide.*

---

11. Click **OK** to save the settings in the Automated Fraction Analysis Settings dialog box.

12. Save the preparative method.

    The Fraction Analysis Settings are saved with the preparative method.

4.4 Running an Automated Analysis

To run an Automated Analysis:

1. Set up the Automated Analysis as described in Section 4.3, Setting Up an Automated Analysis.
2. Open the preparative method.
3. Select **Data Storage** from the Options menu to display the Set Data File Name and Directory dialog box.
4. Specify a **directory** to store the data files that the preparative and analytical methods will create. Type a file name in the **File** text box or click the **Name file when run** check box. Click **OK** to accept the settings and return to the Method Editor.
5. Run the preparative method by selecting **Run** from the File menu, by selecting **Run Multi Method** from the Multi Method menu, or by opening and running a **template**.

   For more information on running multi-methods and templates, see Creating and Running Templates and Multi-Methods, in the *BioCAD 700E Workstation User's Guide*.
6. If you chose to name files when run in the Data Storage options (see step 4), type a **data file name** in the Set Data File Name and Directory dialog box and click **OK**.

   The preparative method collects fractions. The analytical method injects and analyzes the fractions as you specified in the Automated Fraction Analysis Settings dialog box.

NOTE: *Only the first 100 fractions that each preparative method produces are analyzed. If your preparative method produces more than 100 fractions, you can analyze the remainder by running an Analysis template. For more information, see Analysis Template (Integrated Autosampler), in the BioCAD 700E Workstation User's Guide.*

Disabling Automated Analysis

Whenever you run a preparative method that has Automated Analysis settings saved with it, you can disable Automated Analysis. Disabling Automated Analysis allows you to run the preparative method only, without using the Automated Analysis feature.

To disable Automated Analysis, do one of the following:

- If you run a single preparative method, select **Automated Fraction Analysis** from the On Board Analysis menu in the Method Editor so that the check mark disappears.
- If you run a preparative method from a template, deselect the **Automated Fraction Analysis Settings** check box in the template dialog box.

Viewing results

For information on viewing the data files created by the preparative and analytical methods, see the *Data Analysis User's Guide*.

5 Maintenance

This chapter includes the following sections:

For maintenance procedures for the BioCAD 700E Workstation, see Maintenance, in the *BioCAD 700E Workstation User's Guide*.

CAUTION

Only the parts listed in this chapter are recommended for replacement by customers. If you attempt to service any parts not listed in this chapter, you may invalidate your warranty.

5.1 Maintenance Schedule

Regular preventative maintenance will help keep your AFC 2000 functioning properly. Perform the following procedures as indicated:

- **Daily**—Run SYSCLEAN.MET. See page D-7.
- **Weekly**—Clean Z-Rack and sampling needle. See page 5-7.
- **Every six months**—Inspect AFC 2000 syringe seal. See page 5-4.

5.2 Replacing the Fuses

WARNING

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

AVERTISSEMENT

Pour une protection permanente contre le danger d'incendie, remplacez les fusibles existants avec des fusibles similaires, présentant les mêmes caractéristiques.

The AFC 2000 requires two fuses. To replace a blown fuse:

1. Power down the AFC 2000 using the on/off switch on the left side panel of the instrument.
2. Remove the power cord.
3. Power down the BioCAD 700E Workstation.
4. Remove the power cord from the BioCAD 700E Workstation.

5. Pry out the fuse block using a small flat-blade screwdriver (Figure 5-1).

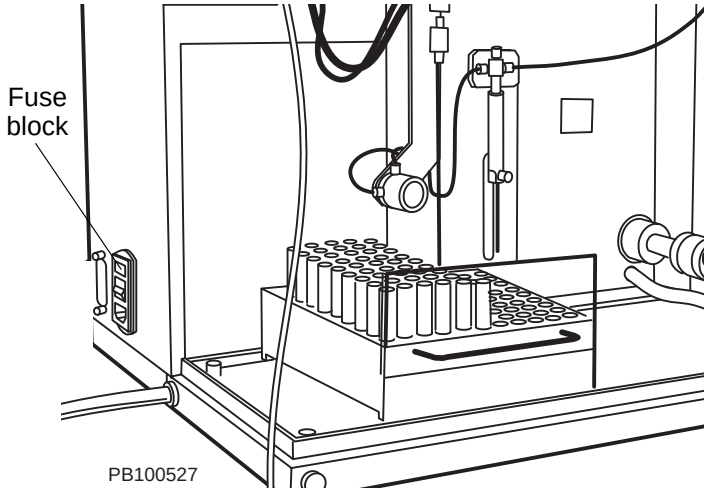


Figure 5-1 Replacing the Fuses

6. Remove the fuse or fuses and replace with a T3.15A 250V fuse.
7. Replace the fuse block.
8. Plug in the power cord and power up the device.
9. Plug in the BioCAD 700E power cord and power up the BioCAD 700E Workstation.

5.3 Inspecting and Changing the AFC 2000 Syringe Seal

Inspect and change (if necessary) the AFC 2000 syringe seal every six months.

The following sections describe:

- Removing the AFC 2000 syringe
- Inspecting the syringe seal
- Changing the syringe seal
- Replacing the AFC 2000 syringe

Removing the syringe

To remove the AFC 2000 syringe:

1. Select **Change Syringe** from the Sample menu in the Sample Table.

   The syringe plunger retracts and the Set Syringe Size dialog box is displayed.

   CAUTION
   Do not remove the syringe before retracting the plunger. The syringe can break.

2. Unscrew and remove the screw located at the bottom front of the plunger.
3. Unscrew and remove the body of the syringe by turning it to the left.

Inspecting the syringe seal

To inspect the syringe seal:

1. Hold the syringe in one hand.
2. Move the plunger through its full range of motion.

   You should feel a constant resistance through the entire range. If the plunger slips at any point, the seal is worn and must be replaced. See “Changing the syringe seal” on page 5-5.

Changing the syringe seal

To change the syringe seal:

1. Remove the syringe as described in “Removing the syringe” on page 5-4.
2. Remove the plunger from the barrel of the syringe.
3. Remove the syringe seal from the top of the plunger by gripping it with a pair of pliers approximately one-third of the way down and pulling it off.
4. Slip a new O-ring over the plunger tip.
5. Wet the O-ring and plunger tip with deionized water.
6. Place the new seal on a flat surface with the open end facing up.
7. Press the plunger tip into the hole in the seal until the seal snaps into place.
8. Wet the seal and insert the plunger into the syringe barrel.
9. Replace the syringe as described in “Replacing the syringe” on page 5-6.

Replacing the syringe

To replace the AFC 2000 syringe:

1. Mount the new syringe on the plunger shaft and pull up on the syringe body until it reaches the fitting (Figure 5-2).

NOTE: *A 500 µl syringe is included.*

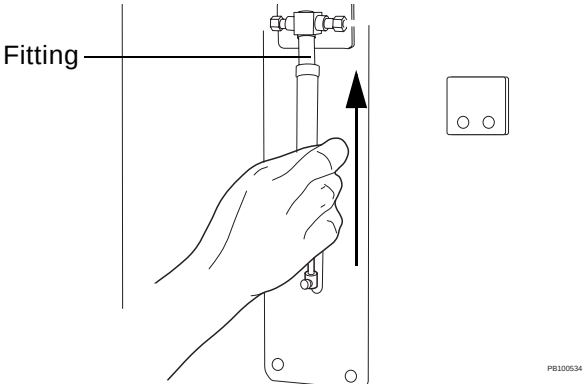


Figure 5-2 Attaching a Syringe to the AFC 2000

2. While pulling up gently, screw the syringe body into the fitting by turning it to the right.
3. Replace the screw at the bottom front of the plunger.
4. Select the **size** of the syringe you installed from the drop-down list box in the Set Syringe Size dialog box. Click **OK**.

   The syringe plunger returns to the home position.

NOTE: *If you attach a 2.5 ml or 5.0 ml syringe, you must also attach an extra length of coiled tubing between the syringe and the sample tubing to contain the larger sample volumes. For more information, see "Attaching coiled tubing" on page 3-9.*

5.4 Cleaning the Sampling Needle

Clean the sampling needle weekly to prevent build-up.

To clean the sampling needle:

1. Power down the AFC 2000 using the on/off switch on the left side panel of the instrument.
2. Wipe the needle gently using a lint-free cloth dampened with isopropanol.
3. Inspect the sampling needle for any cracks or chips. If damaged, replace the sampling needle. See Section 5.6, Replacing the Sampling Needle.

5.5 Cleaning the Z-Rack

The Z-Rack controls the up and down movement of the sampling needle. Clean the Z-Rack weekly to ensure smooth operation of the sampling needle.

To clean the Z-Rack:

1. Power down the AFC 2000 using the on/off switch on the left side panel of the instrument.
2. Wipe the Z-Rack thoroughly with a lint-free cloth.
3. If necessary, use a brush to remove debris from the teeth of the Z-Rack (see Figure 5-3).

CAUTION

Do not use alcohol or solvents to clean the Z-Rack.

5.6 Replacing the Sampling Needle

Replace the sampling needle if the Teflon coating becomes cracked or chipped.

To replace the sampling needle:

1. Power down the AFC 2000 using the on/off switch on the left side panel of the instrument.
2. Loosen the insulation block set screw using a hex wrench (Figure 5-3).

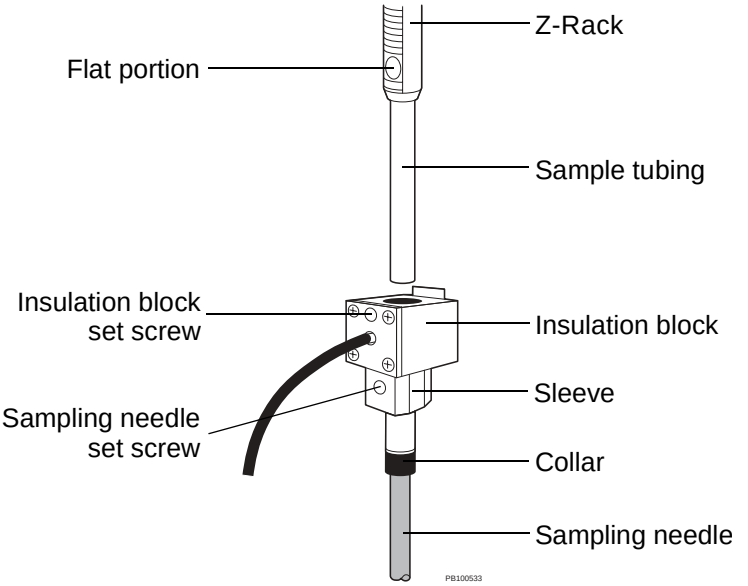


Figure 5-3 Replacing the Sampling Needle

3. Gently pull down on the insulation block until it is approximately 3 inches below the Z-Rack.
4. Remove the sample tubing from the sampling needle by pulling the tubing straight up out of the insulation block.

5. Remove the sampling needle set screw from the insulation block sleeve using a flat screwdriver.
6. Carefully pull the sampling needle straight down out of the insulation block.
7. Slide the non-Teflon coated end of a new sampling needle through the sleeve of the insulation block until the collar reaches the sleeve.
8. Insert the sampling needle set screw into the threaded hole in the insulation block sleeve. Gently tighten the set screw using a flat screwdriver.
9. Slide the sample tubing into the top of the insulation block and over the end of the sampling needle.
10. Attach the insulation block to the Z-Rack, making sure the black coaxial cable is on the left side of the Z-Rack and not twisted.
11. Tighten the insulation block set screw with a hex wrench until it makes contact with the flat portion of the Z-Rack. Do not overtighten the set screw.
12. Align the sampling needle. See “Aligning the Sampling Needle” below.

CAUTION

Failure to align a new sampling needle can damage the needle.

Aligning the Sampling Needle

Align the AFC 2000 sampling needle if you replace the needle or if the needle is not centered over the injection port (see Figure 2-9 on page 2-10). Aligning the sampling needle sets the position of the sampling needle during injections.

For procedures on checking the sampling needle alignment and aligning the sampling needle, see Section 2.8, Checking and Aligning the Sampling Needle.

Troubleshooting and Error Codes

6

This chapter includes the following sections:

6

6.1 Troubleshooting

This section contains troubleshooting information for:

- AFC 2000 Robotic Sample Handling Device
- Sample Table
- Method Editor

NOTE: *For other troubleshooting information, see the BioCAD 700E Workstation User's Guide.*

Technical support

If you are unable to solve problems using the guidelines in the following sections, call Applied Biosystems Technical Support. To reach Applied Biosystems Technical Support, refer to the list of offices on the back cover of this document.

Using modem for remote troubleshooting

If your system includes a modem and remote control software, you can allow Applied Biosystems Technical Support to access your system to diagnose and troubleshoot problems.

For more information, see the *Modem and Remote Control User's Guide*.

6.1.1 AFC 2000 Robotic Sample Handling Device Troubleshooting

This section includes troubleshooting information for the AFC 2000 Robotic Sample Handling Device.

Table 6-1 AFC 2000 Troubleshooting

Symptom	Possible Cause	Action
Integrated AFC 2000 not available in Autosampler/ Fraction Collector section of Configuration dialog box	AFC 2000 is not specified in BioCAD Hardware Setup Program	Update BioCAD Hardware Setup Program. See Section 2.6, Updating the BioCAD Hardware Setup Program.
AFC 2000 does not run startup routine when BioCAD 700E is powered up and BioCAD software is running	AFC 2000 not powered up	Power up AFC 2000 using on/off switch on left side panel of device.
	AFC 2000 is not receiving power	Check power supply and connections.
	Communications failure	Check communications cable between BioCAD 700E Workstation and AFC 2000.
	Hardware fault	Power down and power up AFC 2000. If symptom still present, call Applied Biosystems Technical Support.

Table 6-1 AFC 2000 Troubleshooting (Continued)

Symptom	Possible Cause	Action
AFC 2000 does not respond when you control it from the BioCAD software	Communications failure	Exit and restart the BioCAD software.
		Power down and power up BioCAD 700E Workstation and AFC 2000.
		Call Applied Biosystems Technical Support.
AFC 2000 does not make an injection when instructed to do so by a method or manually	Communication error between computer and AFC 2000	Reset configuration by displaying the Configuration dialog box, then clicking **OK**.
	No samples entered in Sample Table	Enter samples in Sample Table. See Section 3.2.4, Entering Vials in Sample Table.
AFC 2000 stops during an injection	Insufficient volume in vial or insufficient volume entered in configuration	Check volume in vial and check sample vial volume in configuration.
Liquid pools at top of injection port during injections	Sampling needle is not sealing against injection port sleeve	Check sampling needle for scratches or chips and replace if necessary. See Section 5.6, Replacing the Sampling Needle.
		Align sampling needle. See Section 2.8, Checking and Aligning the Sampling Needle.

Table 6-1 AFC 2000 Troubleshooting (Continued)

Symptom	Possible Cause	Action
Method runs, but AFC 2000 does not inject sample	Configuration has incorrect sample vial volume	Check sample vial volume in configuration by double-clicking on a vial position in the Sample Table to display the Vial Editor. Make sure sample vial volume is greater than injection volume set in method. For more information, see Section 3.2.4, Entering Vials in Sample Table.
	Liquid Level Sensing feature is enabled, but sample is nonconductive	Disable Liquid Level Sensing and run method again.
	Level Sensing feature is not working	Call Applied Biosystems Technical Support.
	You used the AFC 2000 to collect fractions earlier in the method and there is no Stop Fraction Collection event in the method	Add a Stop Fraction Collection event to the method before trying to make an injection.
AFC 2000 aspirates sample from the wrong tube when you run a template other than the Analysis template	If the sample volume in the tube you select in the template is insufficient to make the injection, the system automatically injects from another tube with the same sample name that contains sufficient volume (if one exists)	No action. Normal occurrence.

Table 6-1 AFC 2000 Troubleshooting (Continued)

Symptom	Possible Cause	Action
AFC 2000 does not inject an accurate volume	Air bubbles in AFC 2000 solvent line or AFC 2000 syringe	Prime AFC 2000 solvent lines. See Section 3.1.5, Priming the Autosampler Solvent Line.
	Syringe seal in AFC 2000 syringe is not sealing	Replace syringe seal. See Section 5.3, Inspecting and Changing the AFC 2000 Syringe Seal.
	AFC 2000 sampling needle not aligned with injection port	Align AFC 2000 sampling needle with injection port. See Section , Aligning the Sampling Needle.
Air bubbles in AFC 2000 syringe	AFC 2000 solvent bottle is empty	Fill bottle and prime solvent line. See Section 3.1.5, Priming the Autosampler Solvent Line.
	AFC 2000 solvent line is not primed	Prime AFC 2000 solvent line. See Section 3.1.5, Priming the Autosampler Solvent Line.
	Fitting above AFC 2000 syringe is loose	Hand-tighten fitting.
	AFC 2000 syringe is loose	Hand-tighten syringe.
	AFC 2000 syringe is leaking	Change syringe. See Section 5.3, Inspecting and Changing the AFC 2000 Syringe Seal.

Table 6-1 AFC 2000 Troubleshooting (Continued)

Symptom	Possible Cause	Action
AFC 2000 syringe is leaking, and you can turn the syringe barrel without turning the metal band at the top of the barrel	Seal is broken between barrel and metal band	Change syringe. See Section 5.3, Inspecting and Changing the AFC 2000 Syringe Seal.
AFC 2000 arm jam	Rack is misaligned in AFC 2000	Check that rack is seated properly on pins. It may be necessary to remove the rack, then place it back in position. See “Loading racks” on page 3-6.
	Rack configured is different from rack installed	Configure or install different rack. See Section 3.2.1, Configuring as an Integrated Fraction Collector, or Section 3.1.2, Loading Tubes, Plates, and Racks.
	Cables or tubing are obstructing movement of AFC 2000 arm	Untangle and reposition cable or tubing that is obstructing movement of AFC 2000 arm.
	AFC 2000 sampling needle is misaligned with injection port	Align AFC 2000 sampling needle. See Section , Aligning the Sampling Needle.
	Transport bracket not removed from sampling arm or foam piece not removed from Z-Rack slot	Remove transport bracket or foam piece. See “Removing protective packaging” on page 2-4.

Table 6-1 AFC 2000 Troubleshooting (Continued)

Symptom	Possible Cause	Action
AFC 2000 motor does not stop after arm reaches edge of rack	Defective x or y sensor	Call Applied Biosystems Technical Support.
Loud buzzing noise from AFC 2000 syringe area	Defective switch	Call Applied Biosystems Technical Support.
No Fraction Collector Setup command on Config menu	AFC 2000 not configured as fraction collector	Adjust configuration. See Section 3.2.1, Configuring as an Integrated Fraction Collector.
Fraction Collector settings in Control Panel are not used during method run	During a method run, fraction collector settings in method override settings in Control Panel	No action, normal occurrence.
Non-peak fractions are not collected at proper times	Incorrect delay volume set for Fraction Valve to Fraction Collector in Estimate Delay Volumes dialog box	Adjust delay volumes in configuration. See Section 3.2.1, Configuring as an Integrated Fraction Collector.
AFC 2000 is overfilling fraction collection tubes	The rack type and tube size configured is different from the rack type and tube size installed in AFC 2000	Configure the correct rack type. See Section 3.2.1, Configuring as an Integrated Fraction Collector.

Table 6-1 AFC 2000 Troubleshooting (Continued)

Symptom	Possible Cause	Action
When you click the **Advance** button on the Control Panel with an AFC 2000 configured, AFC 2000 does not respond immediately	When the pump is flowing, the AFC 2000 does not advance until the delay volume you specified in the Configuration dialog box is exceeded	No action, normal occurrence.
Fractions not deposited cleanly into tubes	Rack is misaligned in AFC 2000	Check that rack is seated properly on pins. It may be necessary to remove the rack, then place it back in position. See “Loading racks” on page 3-6.
	Cables or tubing are obstructing movement of AFC 2000 arm	Untangle and reposition cable or tubing that is obstructing movement of AFC 2000 arm.
	Outlet of diverter valve is dirty or plugged	Remove PEEK tubing at bottom of diverter valve by loosening fitting. Replace with new piece of PEEK tubing.
		Replace diverter valve.
	The incorrect rack is configured.	Configure the same rack you installed in the AFC 2000. See Section 3.2.2, Configuring as an Integrated Autosampler.

6

6.1.2 Sample Table Troubleshooting

This section includes troubleshooting information for the Sample Table related to blocks, segments, and added events that are available when using the AFC 2000. For additional Sample Table troubleshooting information, refer to the *BioCAD 700E Workstation User's Guide*.

Table 6-2 Sample Table Troubleshooting

Symptom	Possible Cause	Action
AFC 2000 Sample Table does not appear in Window menu	You did not specify AFC 2000 as an integrated autosampler in the BioCAD Hardware Setup Program	Specify AFC 2000 as an integrated autosampler in the BioCAD Hardware Setup Program. See Section 2.6, Updating the BioCAD Hardware Setup Program.
Autoname check box is dimmed in the Vial Editor dialog box	You did not use the Add Vials button to add sample vials to the Sample Table	Click **Add Vials** after selecting vials in the Sample Table. See "Autonaming batch-entered vials" on page 3-36.

6.1.3 Method Editor Troubleshooting

This section includes troubleshooting information for the Method Editor related to blocks, segments, and added events that are available when using the AFC 2000. For additional Method Editor troubleshooting information, refer to the *BioCAD 700E Workstation User's Guide*.

Table 6-3 Method Editor Troubleshooting

Symptom	Possible Cause	Action
AFC 2000 Inject segment button (AI) is dimmed	Sample vials are not entered in the Sample Table	Enter sample vials in the Sample Table. See Section 3.2.4, Entering Vials in Sample Table.
Add Event button is dimmed	A Separation block segment (Step, Gradient, Inject, AFC 2000 Inject, or Purge) is not selected	Select a Separation block segment (Step, Gradient, Inject, AFC 2000 Inject, or Purge).

6

6.2 Error Codes

Error codes may be displayed in warning boxes during normal operation. Error codes are also displayed in the Event Log window.

This section describes the following types of error codes:

- AFC 2000 Robotic Sample Handling Device
- Sample Table
- Method Editor

NOTE: *See the BioCAD 700E Workstation User's Guide for other error codes.*

6.2.1 AFC 2000 Robotic Sample Handling Device Error Codes

Table 6-4 AFC 2000 Error Codes

Error Code	Possible Cause	Action
"AutoSampler Invalid Load Amount"	Injection volume entered in BioCAD software zero or negative value	Type a positive value for injection volume in the AFC 2000 Inject Segment dialog box.
"AutoSampler is Busy"	Autosampler is not ready to perform requested operation	Wait for autosampler to complete current operation before requesting another operation.
"AFC 2000 Device X: Initialization error"	AFC 2000 is not receiving power, hardware fault, or communications failure	• Check power supply, cables, and connections. • Power down and power up AFC 2000. • Exit and restart the BioCAD software. • Power down and power up BioCAD 700E Workstation.
"AFC 2000 Device X: Timeout error"		
"AFC 2000 Device X: Not initialized"		
"AFC 2000 Device X: Offline		

Table 6-4 AFC 2000 Error Codes (Continued)

Error Code	Possible Cause	Action
"AFC 2000 Device 8: No liquid detected"	Liquid Level Sensing is enabled and the sample tube is empty	Fill empty tube.
	Liquid Level Sensing is enabled and tube contains a nonconductive sample that cannot be used with Liquid Level Sensing	Rerun method with Liquid Level Sensing disabled in the AFC 2000 Inject (AI) segment dialog box. See "Adding an AFC 2000 Inject segment" on page 3-57.
"AFC 2000 Device 8: Not enough liquid detected"	Liquid Level Sensing is enabled and volume of sample in tube is insufficient	Fill tube with a sufficient volume of sample.
	Liquid Level Sensing is enabled and Needle depth is too high	Decrease needle depth in the AFC 2000 Autosampler/Fraction Collector dialog box. See "Needle depth" on page 3-22.
"AFC 2000 Device 8: Step loss"	Mechanical obstruction of AFC 2000 arm movement	Remove objects obstructing AFC 2000 arm movement.
	Tubing connected to Z-Rack is catching on frame	Free tubing.
	AFC 2000 sampling needle is misaligned with injection port	Align AFC 2000 sampling needle. See Section , Aligning the Sampling Needle.

6.2.2 Sample Table Error Codes

This section includes error codes for the Sample Table related to blocks, segments, and added events that are available when using the AFC 2000. For additional Sample Table error codes, refer to the *BioCAD 700E Workstation User's Guide*.

Table 6-5 Sample Table Error Codes

Error Code	Possible Cause	Action
"No name has been specified. Enter the name then exit."	You did not enter a Short Name in the Sample Editor dialog box	Type a name in the Short Name text box of the Sample Editor dialog box. See Section 3.2.3, Customizing the Sample Library.
"Cannot edit Sample Table while method is running!"	Method is running	Wait until method finishes, or abort method before editing Sample Table.
"No vial at current location!"	You clicked Load Loop when a blank vial position (no sample loaded) is selected in the Sample Table	Select a vial position in the Sample Table that has a sample loaded (Sample, Standard, Control, or Reagent vial type).
"Invalid vial at current location!"	You clicked Load Loop when an Empty Fraction Ⓕ vial type is selected in the Sample Table	Select a vial position in the Sample Table that has a sample entered (Sample, Standard, Control, Reagent, or Collected Fraction ⓕ vial type). **OR** Add sample to vial and enter vial in Sample Table before clicking **Load Loop**.

Table 6-5 Sample Table Error Codes (Continued)

Error Code	Possible Cause	Action
"Required volume of .. µl exceeds volume in Vial!"	You clicked **Load Loop** when the volume entered in the Volume text box exceeds the volume available in the vial	Type a smaller volume in the Volume text box of the Sample Table.
"Cannot specify zero volume"	You clicked **Load Loop** when the volume entered in the Volume text box is 0.0	Type a value larger than 0.0 in the Volume text box before clicking **Load Loop**.
"Sample has already been loaded into loop"	You are trying to load the sample loop a second time before injecting the contents of the loop	Inject previously loaded sample.
"Injection volume exceeds syringe capacity, amount set to .. µl"	The volume you typed in the Volume text box is greater than the AFC 2000 syringe volume you configured in the AFC 2000 Configuration dialog box	Click **OK** to allow the software to adjust the injection volume.
"Loading .. µl into .. µl loop"	The volume you typed in the Volume text box is greater than the maximum loop volume	Click **Cancel** and adjust the volume. ***NOTE:*** *If you click* ***OK*** *to continue, the system injects the maximum loop volume. It does not inject the volume entered in the Sample Table Volume text box.*

6.2.3 Method Editor Error Codes

This section includes error codes for the Method Editor related to blocks, segments, and added events that are available when using the AFC 2000. For additional Method Editor error codes, refer to the *BioCAD 700E Workstation User's Guide.*

Table 6-6 Method Editor Error Codes

Error Code	Possible Cause	Action
"Method's configuration does not match current System Configuration... Update Method?"	Method configuration does not match system configuration	Load configuration from method or change system configuration.
	Sample names and volumes you entered in the Sample Table do not match the sample names and volumes requested in the method	Change the sample name and volume information in the Sample Table or the method.
"Injection volume exceeds limits, amount set to ...µl"	Injection volume in AFC 2000 Inject segment is greater than the maximum volume that the AFC 2000 syringe can draw	Click **OK** to allow the system to adjust the injection volume.
"Sample Loader: Loading ... µl into ...µl loop."	Injection volume is greater than the loop volume. Injected volume will be smaller than the volume you entered.	Click **Cancel** and adjust the injection volume. ***NOTE:*** *If you click* **OK** *to continue, the system injects a volume less than the volume you entered.*

Table 6-6 Method Editor Error Codes (Continued)

Error Code	Possible Cause	Action
"No <sample> available. Volume set to 0"	Sample listed in method is not listed in the currently loaded Sample Table	Select different sample in AFC 2000 Inject segment, or add sample to Sample Table.
	Sample listed in method has same Short Name as another sample listed in the Sample Table, but has different attributes. These two samples are treated as different samples, even though Short Name is the same.	Select same sample in method and Sample Table. For more information on attributes of a sample, see Section 3.2.3, Customizing the Sample Library.
"<sample> not found"	Sample listed in method is not listed in the currently loaded Sample Table	Select different sample in AFC 2000 Inject segment, or add sample to Sample Table.
"Only .. µl of sample <sample> available"	Not enough specified sample available in sample vial	Adjust injection volume in method, or add sample to vial and adjust volume in Vial Editor dialog box. See Section 3.2.4, Entering Vials in Sample Table.
"Not enough sample available. Vial contains ..µl."		
"Insufficient volume for Loading Template"	Volume in vial not sufficient to run template	Adjust injection volume in method, or add sample to vial and adjust volume in Vial Editor dialog box. See Section 3.2.4, Entering Vials in Sample Table.
"Not enough sample <sample> for Run #... Template canceled."		

Specifications

A

This appendix includes specifications for the AFC 2000 Robotic Sample Handling Device.

Table A-1 General Specifications

Condition	Specification
Weight	~65 pounds (~29 kg)
Dimensions	22 inches (56 cm) wide, 18 inches (46 cm) deep, 19 inches (48 cm) high
Power and fuse requirements	The AFC 2000 Robotic Sample Handling Device requires a grounded outlet for its three-pronged plug. The power and fuse requirements are: • **Power**—110–230 VAC, 47/63 Hz, 150 Watts • **Fuses**—Two T3.15A 250V fuses
Operating temperature	59–104°F (15–40°C)
Fraction collection	AFC 2000 Robotic Sample Handling Device can function as an integrated fraction collector, accommodating various tube sizes. Tube-to-tube switching time is 0.12 seconds when used with the 13 mm test tube rack.

A

Spare Parts

B

This appendix contains part numbers for the following spare parts:

- AFC 2000 fraction collector/autosampler
- Spare Parts Kits

The parts listed in this appendix are recommended for customer installation.

CAUTION

Only the parts listed in this chapter are recommended for replacement by customers. If you attempt to service any parts not listed in this chapter, you may invalidate your warranty.

Procedures other than those listed in Chapter 5, Maintenance, or in the *BioCAD 700E Workstation User's Guide*, Installing the BioCAD 700E Workstation, and Maintenance, may require a trained service representative.

CAUTION

Damage incurred by performing unauthorized work on your system may invalidate your warranty.

Table B-1 AFC 2000 Robotic Sample Handling Device Spare Parts

Item	Where used	Part Number
Cable, RS-232	Left side of AFC 2000	GEN 601090
Plates, Microtiter, 96 well (case of 50)	AFC 2000 rack	GEN 601084 If ordering direct from VWR, use 62409-120
Probe, cap piercing	AFC 2000 arm	GEN 601088
*Rack, Eppendorf (holds 100 2 ml tubes)	AFC 2000 rack area	GEN 601078
*Rack, 50 ml plastic conical tube (holds 25 tubes)	AFC 2000 rack area	GEN 601077
*Rack, 16 mm test tube (holds 90 tubes)	AFC 2000 rack area	GEN 601076
*Rack, 13 mm test tube (holds 100 tubes)	AFC 2000 rack area	GEN 601075
*Rack, Microtiter plate 96 well (holds 2 plates)	AFC 2000 rack area	GEN 601079
Rod, support	For 5 ml coiled tubing on AFC 2000	GEN 601085
Shield, arm, Lexan®	Covers sampling needle and diverter valve on AFC 2000	GEN 601095
Syringe, 500 µl	AFC 2000 syringe pump	P5-1012-00-0005
Syringe, 2.5 ml	AFC 2000 syringe pump	P5-1012-00-0025
Syringe, 5 ml	AFC 2000 syringe pump	P5-1012-00-0050

Table B-1 AFC 2000 Robotic Sample Handling Device Spare Parts (Continued)

Item	Where used	Part Number
*Tubes, Eppendorf, 2 ml (pack of 1,000)	AFC 2000 rack	GEN 601086 If ordering direct from VWR, use 20170-708
*Tubes, 50 ml plastic conical, 26.8 mm ID (case of 500)	AFC 2000 rack	GEN 601082 If ordering direct from VWR, use 21008-714
*Tubes, 16 mm test tube, 14.1 mm ID (box of 1,000)	AFC 2000 rack	GEN 601081 If ordering direct from VWR, use 60825-618
*Tubes, 13 mm test tube, 11.4 mm ID (box of 1,000)	AFC 2000 rack	GEN 601080 If ordering direct from VWR, use 60825-571
Wash station, Teflon	AFC 2000	GEN 601053
***NOTE:** To ensure smooth AFC 2000 operation, use only racks provided by* Applied Biosystems*. Use only tubes and microtiter plates provided by or recommended by* Applied Biosystems*.*		

Table B-2 Spare Part Kits

Kit	Where Used	Part Number
AFC 2000 Plumbing Kit • Plug, blanking (for syringe pump) • Probe, cap piercing • Tubing, fraction, 5 feet, 1/16-inch OD, with 1/8-inch OD sleeve, Teflon (from BioCAD 700E to side of AFC 2000 diverter valve) • Tubing, sample, 51 inches, 1/8-inch OD, Teflon (from left side of syringe to sampling needle) • Tubing, solvent, 2 feet, 1/8-inch OD, Teflon (from solvent to right side of syringe) • Tubing, waste, 55 inches, 1/16-inch OD, with 1/8-inch OD sleeve, Teflon (from top of diverter valve to wash station) • Tubing, waste, 5 inches, 1/16-inch OD, Teflon (from injector valve to wash station) • Tubing, 1/8-inch OD, 5 ml, Teflon, coiled	AFC 2000 Robotic Sample Handling Device	GEN 601072
AFC 2000 Injector Valve Fitting Kit • Ferrules, injector valve (pack of 10) • Fittings, injector valve (pack of 10) • Port, Injection (pack of 2) • Wrench, socket, 1/4 inch • Union, stainless steel	AFC 2000 Injector Valve	GEN 601073

Warranty/Service Information

C

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems such as VISION™, BioCAD®, SPRINT™, or INTEGRAL®, that have built-in computers, installing unauthorized hardware or software may void the Warranty or Service Plan.

C.1 Limited Product Warranty

Limited warranty

Applied Biosystems warrants that all standard components of the COBRA™ AFC 2000 will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its COBRA™ AFC 2000, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on the instrument are themselves warranted to be free of defects in materials and workmanship for ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date of installation, the software designated for use with the product will perform substantially in accordance with the function and features described in its accompanying documentation when properly installed on the product. Applied

Biosystems does not warrant that the operation of the instrument or software will be uninterrupted or error free. Applied Biosystems will provide any software corrections or “bug-fixes” if and when they become available, for a period of ninety (90) days 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 Applied Biosystems 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, and for all other products, the applicable warranty period begins the date the component is received by the buyer.

Warranty exceptions

The above warranties shall not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specification instructions for the product or accessories; performance of improper or inadequate maintenance by the user; installation of software or interfacing not supplied by Applied Biosystems; and modification or repair of the product or the software not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems’ sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed. Such limited warranty is given only to buyer or any third party in the event of use of products furnished hereunder by any third party.

Warranty limitations

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, in tort, warranty, or under any statute (including without limitation, any trade practice, unfair competition, or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person, whether or not foreseeable and whether or not Applied Biosystems 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.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement. Any such affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

C.2 Damages, Claims, Returns

Damages If shipping damage to the instrument is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims After a damage inspection report is secured, Applied Biosystems will supply the replacements and process claims that are initiated by either party.

Returns Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the appropriate address.

BioCAD Sample Methods

D

Sample method description

The BioCAD 700E Workstation includes sample methods, which are stored on the hard drive in the C:\BIOCAD\METHOD directory.

You can use the sample methods:

- For demonstrations and training.
- As templates for creating new methods. Methods are designed so you can substitute different columns or flow rates without affecting the chromatography.

Method configuration

Your system configuration may differ from the method configuration set in the sample methods.

When you open a sample method, a Configuration Mismatch error may be displayed. Click **Update Method with System Configuration** to update the sample method with the current system configuration.

Printing

You can print the sample methods from the Method Editor. All sample methods are stored on the hard drive in the C:\BIOCAD\METHOD directory.

List of sample methods

The following sample methods for the AFC 2000 are included with the BioCAD version 2.05 software and later:

- **APREP.MET**—Preparative method for Automated Analysis feature
- **ANALYT.MET**—Analytical method for Automated Analysis feature or method for use with the Analysis template
- **SYSCLEAN.MET**—Method for cleaning the BioCAD 700E Workstation and the AFC 2000.

NOTE: *When you select an AFC 2000 in the BioCAD Hardware Setup Program, the SYSCLEAN method changes to include events to clean the AFC 2000.*

D.1 APREP.MET—Preparative Method for Automated Analysis

Configuration Configuration settings are listed below:

Instrument Configuration	
High/low pressure	3,000 psi, 0 psi
Buffer/Solvent Channels	• A—Cation Buffer pH 4.5 + 100.0 mM MES/HEPES/Acetate • B—Cation Buffer pH 7.5 + 100.0 mM MES/HEPES/Acetate • C—H_2O • D—NaCl + 3,000.0 mM NaCl
Plumbing	Tandem Columns
Column	Column 1 Position: POROS® HS/M 4.6 mmD/ 100 mmL
Tubing	0.020" (orange)
Flow Cell	Semi-prep
Sample Loader	Autoloader, 5 ml syringe, 2,000 µl loop, 500 µl prime, 100 µl flush
Sample	Sample 1: Cell culture supernatant containing gamma globulin
Fraction Collector	AFC 2000 Rack Definition: Left Rack (A): 13 mm test tube, Right Rack (B): 16 mm test tube
Sample Table	Empty Fractions: A:1–A:100, B:1–B:90
Recorder	Detector Channels UV/Vis #1 and UV/Vis #2

Method settings Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow Rate	3,000 cm/hr (8.31 ml/min)
Initial Column State	Column 1 Inline, Column 2 Bypass
UV/Vis Detector Wavelength	• UV/Vis #1—280 nm • UV/Vis #2—254 nm
Initial Detector State	pH/Conductivity Inline

Block	Includes
Equilibrate	• **Purge segment**—Blend Solvent Channel A (pH 4.5) and Solvent Channel B (pH 7.5) to pH 5.5 and 50 mM, 30 ml/min flow rate, 10 ml volume, Column 1 for Flow Direction at end of Purge • **Step segment**—Equilibrate column for 20 CV with blend of Solvent Channel A and Solvent Channel B to pH 5.5 • **Zero UV Detector event**—Zero UV detector at 20 CV
Load	• **Inject segment**—Inject 2 ml from Autoloader Sample 1 position, Flush Loader
Wash	• **Step segment**—Wash sample onto column with 5 CV blend of Solvent Channel A and Solvent Channel B to pH 5.5 and 50 mM • **Reset Fraction Collector event**—Set at 0 CV
Elute	• **Start Fraction Collection event**—Set at 0 CV • **Gradient segment**—Linear salt gradient for 20 CV from 0 to 500 mM NaCl to elute bound species • **Step segment**—Step to 1 M NaCl and maintain final gradient conditions for 5 CV • **Stop Fraction Collection event**—Set at 4 CV

D.2 ANALYT.MET—Analytical Method for Automated Analysis

Configuration Configuration settings are listed below:

Instrument Configuration	
High/low pressure	3,000 psi, 0 psi
Buffers/Solvent Channels	• E—Water + 0.10% v/v TFA • F—Acetonitrile (ACN) + 0.09% v/v TFA
Plumbing	Tandem Columns
Column	Column 2 Position: POROS R2/H 4.6 mmD/ 100 mmL
Tubing	0.020" (orange)
Flow Cell	Semi-prep
Autosampler	AFC 2000 as an integrated autosampler • Rack Definition: Left Rack (A): 16 mm test tube, Right Rack (B):13 mm test tube • 200 µl loop • 0.5 ml syringe • 5 µl chase volume • 5.0 ml/min injection flow rate • 1.0 ml/min aspiration flow rate • 1 mm needle depth • H_2O as solvent • 500 µl flush volume • 7,500 µl prime volume • 750 µl shallow well wash volume • 2,500 µl deep well wash volume • 25.0 ml/min flush/prime/wash flow rate
Recorder	Detector Channels UV/Vis #1, UV/Vis #2, pH, Conductivity, and Pressure

Method settings Method settings are listed below, including:

- General Settings
- Block/Segment/Added Event Settings

General Settings	
Flow Rate	1,805 cm/hr (5.0 ml/min)
Initial Column State	Column 2 Inline, Column 1 Bypass
UV/Vis Detector Wavelength	• UV/Vis #1—220 nm • UV/Vis #2—254 nm
Initial Detector State	pH/Conductivity Bypass

Block	Includes
Equilibrate	• **Purge segment**—Blend 85% Solvent Channel E and 15% Solvent Channel F, 30 ml/min flow rate, 15 ml volume, Column 2 for Flow Direction at end of Purge • **Step segment**—Equilibrate column for 10 CV with 85% Solvent Channel E and 15% Solvent Channel F • **Zero UV Detector event**—Zero UV detector at 10 CV
Load	• **Autosampler Inject (AI) segment**—Inject 100 µl, Flush Loader, Enable liquid level sensing, Allow partial injections ***NOTE:*** *Select an empty fraction from the Sample list box. When you enable Automated Analysis, the analysis method will automatically analyze all fractions that meet the analysis settings that you specify in the Automated Fraction Analysis Settings dialog box.*
Wash	• **Step segment**—Wash sample onto column with 2 CV blend of 85% Solvent Channel E and 15% Solvent Channel F
Elute	• **Gradient segment**—Linear gradient for 15 CV, from blend of 85% Solvent Channel E and 15% Solvent Channel F to blend of 10% Solvent Channel E and 90% Solvent Channel F • **Step segment**—Maintain final gradient conditions for 5 CV

D.3 SYSCLEAN.MET—Shutdown

Configuration

The SYSCLEAN.MET method is for automated system cleaning and shutdown. However, the method does not include steps to clean inline columns. All flow is in the bypass column mode. Set High Pressure limit to 3,000 psi.

CAUTION

The system must be in Single Column or Tandem Column plumbing configuration to run SYSCLEAN.MET. You cannot bypass columns in other plumbing configurations.

Method settings

To run this method, place all solvent and sample lines in a beaker of filtered deionized water (or other appropriate flush solution). Place a tube containing filtered deionized water in the AFC 2000 rack, position A:1. Place empty tubes in positions A:2 through A:10.

Method settings are listed below:

General Settings	
Flow rate	0 ml/min
Initial Column State	Columns bypassed
UV/Vis Wavelength	254 nm
Initial Detector State	pH/conductivity bypassed

Block	Includes
Clean	• **Step segments A through F**—Flushes all buffer/solvent lines (A–F). • **Set SCOUT Column events**—Flushes SCOUT lines (1–6) and valve. • **Load Sample into Loop events**—Flushes autoloader sample lines (1–4) and injection valve. • **AFC 2000 events**—Flush injector and diverter valves on AFC 2000. • **Turn UV Detector Lamp Off event**—Turns off UV/Vis detector.

Technical Support and Training

E

This appendix contains the following sections:

E

E.1 Contacting Technical Support

Overview

You can contact Applied Biosystems for technical support:

- By e-mail
- By telephone or fax
- Through the Applied Biosystems web site

NOTE: *For information on obtaining technical documents such as Applied Biosystems user documents, MSDSs, and certificates of analysis,* *see "Obtaining Technical Documents" on page E-8.*

By E-mail

You can contact technical support by e-mail for help in the product areas listed below.

Product/Product Area	E-Mail Address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com
• Biochromatography • Expedite™ (8900) DNA Synthesis System • PNA • Pioneer™ Peptide Synthesis System • Proteomics Solution 1™ (PS1) System • ICAT™ reagent • FMAT™ 8100 HTS System • Mariner™ Mass Spectrometers • Voyager™ Mass Spectrometers • CytoFluor® 4000 Fluorescence Plate Reader	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

By telephone or fax (North America)

To contact Applied Biosystems Technical Support in North America, use the telephone or fax numbers in the table below.

NOTE: *To schedule a service call for other support needs, or in case of an emergency, dial* ***1.800.831.6844, then press 1****.*

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1.800.831.6844**, then press **8**[a]	1.650.638.5981
DNA Synthesis	**1**.**800**.**831**.**6844**, press **2**, then press **1**[a]	1.650.638.5981
Fluorescent DNA Sequencing	**1**.**800**.**831**.**6844**, press **2**, then press **2**[a]	1.650.638.5981
Fluorescent Fragment Analysis (including GeneScan® applications)	**1**.**800**.**831**.**6844**, press **2**, then press **3**[a]	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1**.**800**.**831**.**6844**, press **2**, then press **4**[a]	1.650.638.5981
ABI PRISM® 3100 Genetic Analyzer	**1**.**800**.**831**.**6844**, press **2**, then press **6**[a]	1.650.638.5981
Peptide Synthesis (433 and 43*x* Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **1**[a]	1.650.638.5981
Protein Sequencing (Procise® Protein Sequencing Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **2**[a]	1.650.638.5981

Product/Product Area	Telephone	Fax
PCR and Sequence Detection	**1**.**800**.**762**.**4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI PRISM® 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1**.**800**.**831**.**6844**, then press **5**[a]	1.240.453.4613
• Voyager™ MALDI-TOF Biospectrometry Workstations • Mariner™ ESI-TOF Mass Spectrometry Workstations • Proteomics Solution 1™ (PS1) System • ICAT™ reagent	**1**.**800**.**899**.**5858**, press **1**, then press **3**[b]	1.508.383.7855
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1**.**800**.**899**.**5858**, press **1**, then press **4**[b]	1.508.383.7855
Expedite™ (8900) Nucleic Acid Synthesis Systems	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855
PNA Custom and Synthesis	**1**.**800**.**899**.**5858**, press **1**, then press **5**[b]	1.508.383.7855

Product/Product Area	Telephone	Fax
FMAT™ 8100 HTS System CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	1.508.383.7855
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	1.781.275.8581
LC/MS (Applied Biosystems/MDS Sciex)	**1.800.952.4716**	1.508.383.7899

a. 5:30 A.M. to 5:00 P.M. Pacific time.

b. 8:00 A.M. to 6:00 P.M. Eastern time.

c. 9:00 A.M. to 5:00 P.M. Eastern time.

By telephone or fax (outside North America)

To contact Applied Biosystems Technical Support or Field Service outside North America, use the telephone or fax numbers below.

Region	Telephone	Fax
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	61 3 9730 8600	61 3 9730 8799
China (Beijing)	86 10 64106608 or 86 800 8100497	86 10 64106617
Hong Kong	852 2756 6928	852 2756 6968
Korea (Seoul)	82 2 5936470/6471	82 2 5936472
Malaysia (Petaling Jaya)	60 3 79588268	603 79549043
Singapore	65 896 2168	65 896 2147
Taiwan (Taipei Hsien)	886 2 2358 2838	886 2 2358 2839
Thailand (Bangkok)	66 2 719 6405	662 319 9788

Region	Telephone	Fax
Europe		
Austria (Wien)	43 (0)1 867 35 75 00	43 (0)1 867 35 75 11
Belgium	32 (0)2 532 4484	32 (0)2 582 1886
Denmark (Naerum)	45 45 58 60 00	45 45 58 60 01
Finland (Espoo)	358 (0)9 251 24 250	358 (0)9 251 24 243
France (Paris)	33 (0)1 69 59 85 85	33 (0)1 69 59 85 00
Germany (Weiterstadt)	49 (0) 6150 101 0	49 (0) 6150 101 101
Italy (Milano)	39 (0)39 83891	39 (0)39 838 9492
Norway (Oslo)	47 23 12 06 05	47 23 12 05 75
Portugal (Lisboa)	351.(0)22.605.33.14	351.(0)22.605.33.15
Spain (Tres Cantos)	34.(0)91.806.1210	34.(0)91.806.12.06
Sweden (Stockholm)	46 (0)8 619 4400	46 (0)8 619 4401
Switzerland (Rotkreuz)	41 (0)41 799 7777	41 (0)41 790 0676
The Netherlands (Nieuwerkerk a/d IJssel)	31 (0)180 392400	31 (0)180 392409 or 31 (0)180 392499
United Kingdom (Warrington, Cheshire)	44 (0)1925 825650	44 (0)1925 282502
European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	27 11 478 0411	27 11 478 0349
Africa, French speaking (Paris, France)	33 1 69 59 85 11	33 1 69 59 85 00
India (New Delhi)	91 11 653 3743 91 11 653 3744	91 11 653 3138
Poland, Lithuania, Latvia, and Estonia (Warszawa)	48 22 866 4010	48 22 866 4020

Region	Telephone	Fax
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia	44 1925 282481	44 1925 282509
Japan		
Japan (Hacchobori, ChuoKu, Tokyo)	81 3 5566 6230	81 3 5566 6507
Latin America		
Caribbean countries, Mexico, and Central America	52 55 35 3610	52 55 66 2308
Brazil	0 800 704 9004 or 55 11 5070 9654	55 11 5070 9694/95
Argentina	800 666 0096	55 11 5070 9694/95
Chile	1230 020 9102	55 11 5070 9694/95
Uruguay	0004 055 654	55 11 5070 9694/95

Through the Applied Biosystems web site

To contact Technical Support through the Applied Biosystems web site:

1. Go to **www.appliedbiosystems.com**
2. Click **Services & Support** at the top of the page, then click **Frequently Asked Questions**.
3. Click **Contact Support** in the contents list at the left of the screen.
4. Click your geographic region for the product area of interest.
5. In the Personal Assistance form, enter the requested information and your question, then click **Ask Us RIGHT NOW**.
6. In the Customer Information form, enter the requested information, then click **Ask Us RIGHT NOW**.

Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

E

E.2 Obtaining Technical Documents

Overview

You can obtain technical documents, such as Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents for free, 24 hours a day. You can obtain documents:

- By telephone
- Through the Applied Biosystems web site

Ordering documents by telephone

To order documents by telephone:

1. From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2. Follow the voice instructions to order documents (for delivery by fax).

NOTE: *There is a limit of five documents per fax request.*

Obtaining documents through the web site

To view, download, or order documents through the Applied Biosystems web site:

1. Go to **www.appliedbiosystems.com/**
2. At the top of the page, click **Services & Support** at the top of the page, then click **Documents on Demand**.
3. In the search form, enter and select search criteria, then click **Search at the bottom of the page**.
4. In the results screen, do any of the following:
   - Click  to view a PDF version of the document.
   - Right-click  , then select **Save Target As** to download a copy of the PDF file.
   - Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you.
   - Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you.

NOTE: *There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.*

E.3 Obtaining Customer Training Information

To obtain Applied Biosystems training information, go to **www.appliedbiosystems.com**, click **Services & Support** at the top of the screen, then click **Training**.

E

Index

A

B

C

INDEX

D

E

F

G

H

I

L

M

N

O

P

R

S

T

U

V

W

Z

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

Worldwide Sales and Support
Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our web site at www.appliedbiosystems.com or to the Technical Support and Training appendix in this document.

www.appliedbiosystems.com

Applied Biosystems

Applera Corporation is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Printed in the USA, 08/2001
Part Number 8-0189-40-0597 Rev. A

an **Applera** business