# Gilson FC 203B Fraction Collector User's Guide



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# **Declaration of Conformity**

Application of Council Directives:

89/336/EEC, 73/23/EEC

Standards to which Conformity is Declared:

EN61326, EN61000-3-3, EN61000-3-2, EN61010-1

Manufacturer's Name ...... Gilson, Inc.

EC Office Address .......Gilson S.A.S.

19 Avenue des Entrepreneurs, B.P. 145 F-95400 Villiers-le-Bel, France

Type of Equipment ......Laboratory Equipment

Model. FC 203B Fraction Collector

Beginning with Serial Number: 179A6001

Month and Year of Manufacture: January 1996

I, the undersigned, hereby declare that the equipment specified above conforms to the above Directives and Standards.

Place: Middleton, WI (USA) Issue Date: January 1996 Michael Jacquart Senior Vice President

Corporate Technology Development



Welcome to improved fraction collecting. The Gilson FC 203B Fraction Collector is simple to use and straightforward to learn. Its sophisticated design makes it flexible enough to adapt to your collection goals. The fraction collector is designed to make every operation clear and efficient.

You can get information about the fraction collector from these sources:

- The **HELP** key on the 203B's keypad offers specific, on-line instructions during setup and operation.
- The 203B Quick Reference Guide answers key points quickly. Two copies are included.
- This **User's Guide** describes how to operate the 203B.
- Gilson's customer service team is available to answer your questions. Refer to page 1-3 for contact information.

**Note**: If you're controlling the FC 203B Fraction Collector via Gilson UniPoint™ System Software, you can obtain the following technical notes from your Gilson representative: Fraction Collector Control (part number LT21373) and Large-Volume Preparative System Control Using a 30x Pump for Repetitive Injection (part number LT21375).



### **Unpacking**

Unpack the fraction collector and accessories carefully from the carton. Cross-check the contents against the standard equipment checklist shown below and against your purchase order's optional accessory list to verify that all parts are included and undamaged.

Do this now, even if your fraction collector will not be used immediately. Many carriers must receive concealed damage claims within seven days of delivery.

The fraction collector is shipped with its drain trough and dispense head already installed.

Your standard accessories include the following:

- rack holder assembly (with antlers)
- 80-cavity collection plate
- two Code 1 racks (for 80, 13 x 100 mm tubes)
- tube retaining bar (for Code 1 rack)
- inlet tubing, 0.031" (0.8 mm) ID, with two Omnifit<sup>TM</sup>-type end fittings
- polypropylene pan
- terminal block connector
- spare fuse
- power cord
- FC 203B Fraction Collector User's Guide
- FC 203B Fraction Collector Quick Reference Guide (2 copies)
- tube gauge

If you ordered a diverter valve with the fraction collector, the standard accessory package also includes a 3-way valve kit.

Please retain all packing material so unit may be shipped safely, if necessary.

#### **Customer Service**

Gilson, Inc. and its worldwide network of authorized representatives provide customers with four basic types of assistance: sales, technical, applications, and instrument repair.

If you need assistance, please contact your Gilson representative or if you are in the United States call the Gilson Customer Service Department at 800-445-7661 or 608-836-1551. You can also contact the Gilson Customer Service Department via its e-mail address: service@gilson.com. Specific contact information can be found on the Gilson web site at www.gilson.com. To help us serve you quickly and efficiently, please refer to the **Before calling us** section on page 5-7.

# **Technical Specifications**

Warning: Changes or modifications to the fraction collector not expressly approved by Gilson could void your factory-authorized warranty.

The fraction collector has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC commercial environment. The fraction collector generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. Operation of the fraction collector in a residential area is likely to cause harmful interference; in which case, the user will be required to correct the interference at the user's own expense.

Shielded cables must be used with the fraction collector to ensure compliance with the Class A FCC limits.

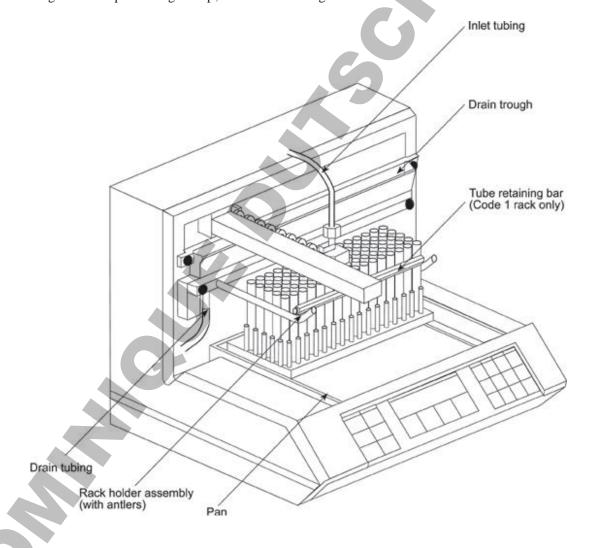
Technical Specification	Definition
3-way valve	Three-port, PTFE, 67 µL internal volume, 3.5 µL dead volume from the common port to the normally closed port, up to 20 mL/minute with standard or large bore needle, up to 20 psi (1.4 bar)
Contact control	Contact closure inputs for start/advance and end/home Event mark output One programmable output for control of peripheral devices
Data acquisition	An input channel for analog-to-digital conversion; digital data is transmitted on the GSIOC
Detector input	±100 mV full scale
Display	Two lines of 24 alphanumeric characters; backlit LCD
Drop counting	Up to 9999 drops per fraction. Maximum rate: 20 drops per second
Environmental conditions	Indoor use Altitude: up to 2000 m Temperature range: 5°-40°C Air pressure: 75-105 kPa Pollution degree: 1 or 2, in accordance with IEC 66 Humidity: Maximum relative humidity 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C
Event marker	100 ms pulse (contact closure)
Front panel	Five command hard keys, HELP, YES, and NO hard keys, 0-9 numeric hard keys, and four soft keys
Manufacturing standards	Meets applicable Safety and EMC certification standards; UL and CE certified
Maximum collection volume/tube	20 mL (28 x 60 mm scintillation vials with optional Code 24 rack)
Maximum fractions	128 (12 x 75 mm tubes with optional Code 14 rack)

Multi-cycle operation	Repetitive collection of each sample into same set of tubes or collection each sample into different set of tubes
Multiple column collection	Simultaneous collection from up to eight columns with installation of optional multiple column adapter
Number of racks	Holds one rack, see Appendix A for available racks
Operating modes	Time, drop, peak + time, peak + drop; and manual
Peak detection	Adaptive slope algorithm that applies user-specified parameters to accommodate drifting baselines, negative peaks, and asymmetrical peaks or absolute threshold level that collects all peaks above specified millivolt value
	Peak parameters: peak height or level, in mV, and peak width at half-height, in minutes
	Minimum peak height for fractionation: 0.1 mV full scale
	Detector-collector delay: 0.01 minute increments
Physical space requirement	32.4 x 29.2 x 26.7 cm (12.75 x 11.5 x 10.5 in.)
Power requirements	Frequency: 50 to 60 Hz  Voltage: 100-120 V or 220-240V, mains voltage fluctuations not to exceed ±10% of the nominal voltage  Current rating: 0.5A for 100-120V or 0.25A for 220-240V
Programmable time units (min.)	From 0.01 to 99.99 minutes per tube, with 0.01 minute limit of resolution
Software control	Via Gilson Serial Input/Output Channel (GSIOC) or by contact closure
Time based programming	Up to ten collection windows and ten drain steps in any mode
Tube change time	100-250 ms, center-to-center, depending on rack type
Weight	5 kg (11 lbs.)
Zero-power memory	Maintains memory for a minimum of five years from date of shipment



# **Plumbing Setup**

Throughout the plumbing setup, refer to the diagram below.



#### **Hardware**

#### Pan

Set the pan in place so it rests on the floor of the fraction collector. Note that the pan will fit properly only when positioned with its beveled edge facing the front of the instrument.

#### Rack holder

Secure the rack holder with two thumbscrews (provided) in the upper position for the standard Code 1 rack (for 13 mm tubes) and for Code 0, 2, 4, 11, and 14 racks.

If you are using a Code 30, 32 (with 13 x 65 mm tubes), or 33 rack, you must install the special rack holder designed for thermostated racks.

Do not install the rack holder when using Code 15, 20, 21, 23, 23W, 24, 28, 29, 29 LE, or 29 SE racks.

#### Rack

Set the rack in place.

Most tubes with a maximum 13 mm outer diameter (OD) will fit the Code 1 rack. Gilson recommends Kimble #73500 or Corning #99445 test tubes.

Use the tube gauge located in the accessory package to check the diameter of your tubes. If a tube does not slide through the tube gauge, it's too big to fit a Code 1 rack.

You'll need a different rack if other size tubes will be used. Look in *Appendix A* for information about other racks.

**Note**: Code 0, 2, 4, 11, and 14 racks fit on the antlers of the rack holder. To install one of the stainless steel racks that fit on the antlers, see page A-3.

The Code 15 microplate holder attaches with thumbscrews to the back face of the fraction collector. If you are using a standard microplate, attach the Code 15 plate holder in the upper position. If you are using a deep-well microplate, attach the Code 15 plate holder in the lower position.

Code 1, 20, 21, 23, 23W, 24, 28, 29, 29 LE, and 29 SE racks fit directly in the pan.

Code 30, 32 (with 13 x 65 mm tubes), and 33 racks fit on the special rack holder for thermostated racks.

Code 32 rack (when used with 13 x 100 mm tubes) fits directly in the pan.

#### **Tube retaining bar**

When using the Code 1 rack (for 13 x 100 mm tubes), set the tube retaining bar in place, as shown on page 2-1. This bar is required to keep the tubes in an even rectangular pattern.

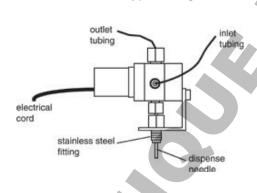
# outlet tubing

COMM

dispense



Omnifit™-type Fitting



#### 3-Way Valve Installation

If you purchased a 3-way valve with your fraction collector, you can install it now. A 3-way valve is highly recommended for collection when using collection windows. It is required if you are collecting into a microplate installed on a Code 15 plate holder.

The 3-way valve prevents spillage when effluent is diverted to waste and simplifies collection of pure peaks.

**Note**: The 3-way valve operates at flow rates up to 20 mL/min. and pressures up to 20 psi. The dead volume of the 3-way valve is 3.5  $\mu$ L from the common (COMM) port to the normally closed (NC) port. Refer to the diagram on the left.

Before installing the 3-way valve, turn off power to the fraction collector using the rear panel power switch.

Gently pull the dispense head away from the drain trough.

#### Inlet tubing

From the valve's accessories, locate the inlet tubing that has fittings on both ends.

Using the illustration at left for reference, attach one end of the inlet tubing to the valve source inlet. To do this, you'll have to remove the plastic nipple from the 3-way valve.

Fingertighten the fitting. Pull gently on the tubing to make sure the fitting is firmly seated.

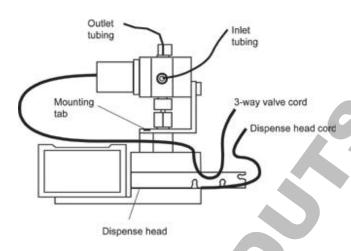
Connect the other end of the inlet tubing to the outlet of your detector. It is good practice to keep the length of tubing from the detector outlet to the dispense head as short as possible. But remember to leave enough tubing to account for movement of the dispense head.

#### **Outlet connection**

The outlet tubing is already connected to the 3-way valve. Place the other end of the outlet tubing into a drain or waste receptacle.

#### **Assembly**

You are now ready to install the assembly on to the top of the dispense head. Use the drawing below for reference.

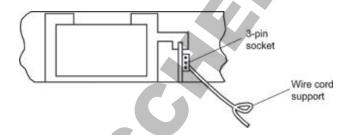


Remove any connections to the top of the dispense head. Note the small mounting tab on the drop detector cover next to the threaded female fitting. When properly installed, this tab protrudes through the hole on the L-bracket of the 3-way valve.

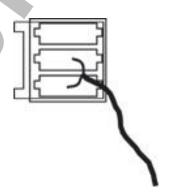
Install the 3-way valve by fingertightening the stainless steel fitting into the dispense head. You may encounter some resistance after a few turns. If so, use a 5/16" open-end wrench to completely tighten the valve. Do not over-tighten; tighten only until the assembly is stable.

#### **Electrical connection**

Now make the electrical connection for the 3-way valve. Locate the socket in the fraction collector into which you will insert the plug from the 3-way valve. To do this, follow the dispense head's spiral cord to the inside of the fraction collector. Just to the right of the cord, you'll see a socket containing three pins.



Note that the plug from the valve has two small tabs on one side. Insert the plug into the socket with the two tabs facing toward the left.



The fraction collector is now ready for operation with a 3-way valve. Once the electrical connection is made, the fraction collector knows the 3-way valve is in place and acts accordingly. The dispense head will not travel to the drain trough during drain periods but is always located over the current tube. Effluent is diverted to drain through the 3-way valve's outlet tubing.

The fraction collector automatically detects the presence of the 3-way valve. The instructions you give to the fraction collector are the same, whether or not the 3-way valve is installed.

**Note**: In the event of power failure, effluent is diverted to drain and is not collected.

#### **Tubing**

#### **Inlet tubing**

**Note**: If you installed a 3-way valve, you've already connected the inlet tubing to the fraction collector and the detector. Proceed with **Drain trough connection** on the next page.

Using the diagram, locate the inlet tubing. It has Omnifit<sup>TM</sup>-type fittings on both ends.



Omnifit™-type Fitting

One end of the inlet tubing extends 11/32" beyond the fitting. Screw this end into the threaded opening at the top of the dispense head. Fingertighten only.

**Note**: The length of tubing that extends from the fitting is critical to accurate drop collection. If the tip becomes damaged, use the tubing gauge described in *Appendix E* to position the fitting on the replacement tubing.

The ID of the inlet tubing supplied with the fraction collector is 0.031" (0.8 mm).



#### **Detector connection**

If a detector is connected to your fraction collector, it is good practice to keep the length of tubing from the detector outlet to the dispense head as short as possible.

**Note**: Remember to leave enough tubing to account for movement of the dispense head.

The outlet tubing from Gilson detectors can be directly coupled to the fraction collector's inlet tubing, since both have Omnifit<sup>TM</sup>-type fittings at their ends. Use the 1/4-28 coupler that came in your detector's accessory package. If necessary, a package of five couplers can be ordered from Gilson (part number F1410050).

#### **Drain trough connection**

Cut the drain tubing supplied with the fraction collector into two appropriately sized pieces. Connect one end of a piece of tubing to the drain port of the drain trough and place the other end into a drain or container.

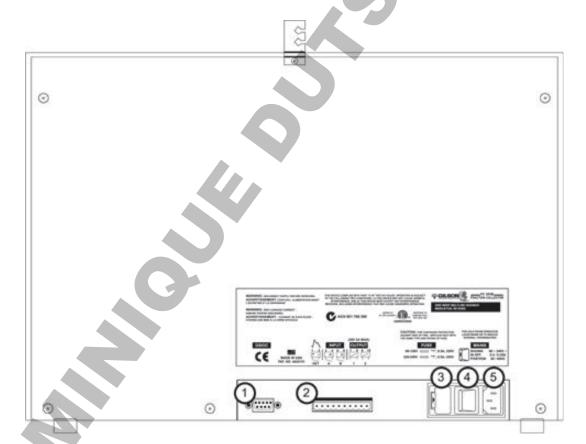
# **Electrical Setup**

#### **Rear Panel Description**

Connections on the FC 203B Fraction Collector's rear panel include:

- 1 Gilson Serial Input/Output Channel (GSIOC) port
- 2 Input/Output (I/O) port
- 3 Fuse drawer
- 4 Power switch
- 5 Power receptacle

**Note**: Please read this entire section and refer to the rear panel diagram below before making any electrical connections. Do not attach the fraction collector's power cord until all other connections have been made.



Rear Panel Diagram

#### Input/Output (I/O) Port

You can take advantage of the powerful built-in features of your fraction collector when you connect other equipment to the multipurpose I/O port.

The port has 10 contacts, numbered 1–10. All of the connections are paired contact closures (no voltage) except for the DET (detector) input which requires an analog detector signal ( $\pm 10$  mV to  $\pm 100$  mV).

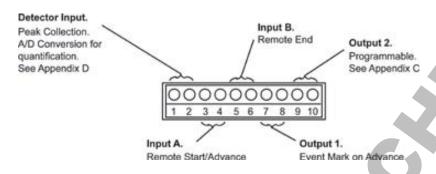
TTL signals can be used as long as the voltage is no greater than 5V.

Some uses for the I/O contact pairs are listed below.

- When you attach a detector to the DET input (1-2 pair), the 203B can collect peak-containing fractions. Data from the detector can be digitized and sent to a controller for quantification of peaks. This input was zeroed at the Gilson factory.
- When you connect a remote switch contact to Input A (3-4 pair), other equipment can signal the 203B to begin collection and to advance the dispense head.
- When you connect a remote switch contact to Input B (5-6 pair), other equipment can signal the 203B to end collection.
- When you connect your chart recorder's event mark input to Output 1 (7-8 pair), you can indicate tube advance on the chart.
- When you connect another device to programmable Output 2 (9-10 pair), the 203B can automatically start and stop that device.

In the section called **Wiring to Other Equipment**, you will find information about how to make these I/O connections work for you.

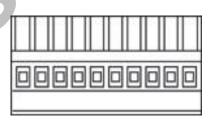
The diagram below shows the location of the multipurpose I/O contacts. Make the I/O connections to the terminal block connector that attaches directly to the fraction collector. You will find detailed installation instructions in the next section.



#### Items you'll need

To make connections to the multipurpose I/O port, you'll need:

- terminal block connector (part number 638310512) included with your fraction collector accessories
- 2-conductor interconnect cable for each connection you'll make. Use any 2-conductor cable with a gauge from 22–30G for each wire. You can purchase a 6-foot piece of suitable cable (part number 709910206) or a package of five cables with identification markers (part number 36078155) from Gilson.
- wire insulation stripper
- small-blade screwdriver
- additional items for connection to other devices; they'll be specified in the text



#### **Making connections**

To prepare wire for each connection, cut it into pieces of appropriate length; then strip about 1 cm of insulation from each end.

Insert each wire into the appropriate slot on the terminal block connector. Push each wire all the way in; then tighten the corresponding pin screw.

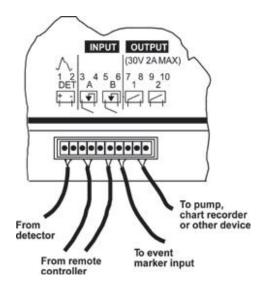
**Note**: Be sure to maintain the correct orientation of the terminal block connector relative to the multipurpose I/O port. With the pin screws facing you and the wires entering from the bottom, the DET input is at the far left.

**Note**: Attach all wires before joining the terminal block connector to the multipurpose I/O port on the fraction collector's rear panel.

Connect the terminal block connector with the wires facing down and the pin screws facing away from the 203B. This prevents spillage from dripping onto the contacts.

Push the terminal block connector as far as it will go. The block is designed to fit snugly into its receptacle.

A configuration using all five inputs and outputs would look like the diagram below.



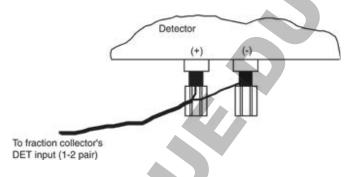
#### Wiring to Other Equipment

#### **Detector input**

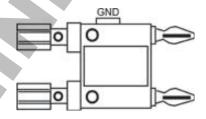
To separate peaks as they elute from your column, a detector is required. When the DET input (1-2 pair) of your fraction collector is linked to a detector, you can specify whether you want to collect peak fractions or peak and non-peak fractions.

Detectors generally have one of two types of output connectors. Using the accompanying figures, determine which output connectors are used on your detector.

One type of detector has screwable binding posts built into the rear panel of the detector. To attach the fraction collector to these outputs, connect lead 1 to the "+" post on the detector and lead 2 to the GND post. Tighten the posts securely. If you're not using a Gilson detector, refer to the Note and diagram on the next page.

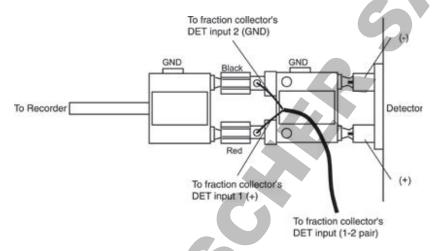


The other type of connector, like the one on most Gilson detectors, has fixed binding receptacles. To attach this type, you'll need a Pomona cable adapter (part number 6374022611).

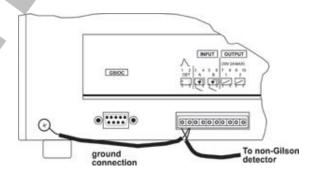


**Pomona Cable Adapter** 

Make the connections to your fraction collector as shown below. Be sure to connect lead 1 to "+" and lead 2 to GND.



Note: If you're using a non-Gilson detector, you may need to make another connection to reduce ground noise. Connect a wire between the DET input 2 (GND) and the metal screw in the lower left or right corner of the fraction collector. This ensures that the detector is properly grounded. If the detector is not grounded, collection problems may occur as a result of the different electrical references for the fraction collector and detector.



Now you're ready to collect fractions in one of the peak detection modes. You're also set up to convert the peak information into digital data for quantification by the Gilson HPLC system control software. See *Appendix I* for more information.

#### Remote start/advance and remote end inputs

As an alternative to front panel control of starting, advancing, and ending, you can control these functions remotely:

- Input A (3-4 pair) controls remote start and advance.
- Input B (5-6 pair) controls remote end.

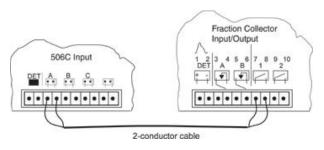
When making the Input A and Input B connections, the source must provide either contact closure (no voltage) signals between "+" and GND or a correctly polarized TTL (0, 5V) compatible signal (0 activates input).

Full-function remote control can be achieved through the GSIOC port. See *Appendix F* for details,

#### Event mark output at tube advance

You can mark tube advance directly on your data chart using the FC 203B Fraction Collector event mark output (Output 1, 7-8 pair). The connections you make for event marks depend on the data unit in the system. Refer to the appropriate section below.

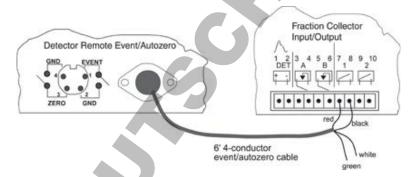
Gilson 506C System Interface and 712 HPLC
System Control Software. In systems with a Gilson 506C System Interface, use a 2-conductor cable to make the event mark connection.
Connect the fraction collector event mark output to Input A of the 506C. In the Contact Events box of the Gilson 712 HPLC System Control Software, label Input A as the "FC 203B Event Mark," but don't enter any timed events for this input. In the Contact dialog of a UniPoint control method, label the contact and choose the Event check box.



Event mark connection between fraction collector and 506C

Gilson Detectors. In systems with a detector as the data unit, use the event/autozero cable (part number 631004021) supplied with the detector to make the event mark connection. The black and red leads on this cable attach to the fraction collector event mark output (7-8 pair). Attach either wire to either slot.

Note: If you are collecting in a peak mode, do not attach the event mark output pair to the detector. Instead, connect the event mark output pair directly to your chart recorder or integrator. This will prevent the event mark being interpreted as a peak.



Event Mark Connection between fraction collector and detector

Non-Gilson data unit (e.g., chart recorder, integrator, non-Gilson software) or Gilson 305 Pump. In these systems, use 2-conductor cable to connect the 203B event mark output to the appropriate input pair on the data unit. If your data unit cannot accept input from the fraction collector, you may connect the fraction collector event mark output pair to the appropriate event input pins of your detector. When set up this way, the data unit may interpret event marks as negative peaks that will appear on the actual detector trace.

**Note**: The 305 does not accept the input from the fraction collector event mark output. Therefore, connect the fraction collector event mark output to a chart recorder, integrator, or detector.

**Note:** The event input pins on the Kipp & Zonen BD 11E/12E recorders available from Gilson, as well as most other Kipp & Zonen recorders, can accept input from the fraction collector event mark output.

#### Event mark output at peak start

If you are collecting in peak mode, you can get an event mark at the start of a peak, in addition to the event mark that indicates tube advance at the end of a peak.

- Make the event mark at tube advance connection between the 203B and your data unit as described above.
- Short Output 2 (9-10 pair) to Output 1 (7-8 pair) of the 203B.
- Using the Technical menu (EDIT 2), select the Out 2 ON at peak start option for programmable Output 2. See the next section for details on programmable Output 2.

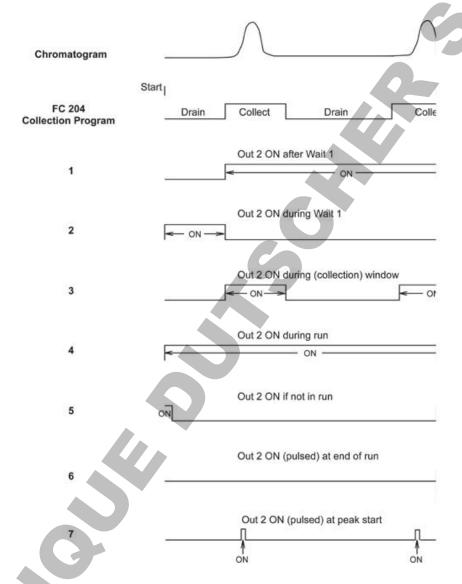
#### **Programmable output**

The fraction collector has several jobs to accomplish during normal operation. It may be waiting, draining, or collecting.

Programmable Output 2 (9-10 pair) can direct other equipment (a chart recorder or peristaltic pump, for example) to be active only when the fraction collector is doing one of those jobs. By selecting one of the Output 2 options, you can instruct a device to be:

- 1 ON after an initial wait period (Wait 1)
- 2 ON during an initial wait period (Wait 1)
- 3 ON during a collection window
- 4 ON during an entire run (wait, drain and collect)
- 5 ON only when the 203B is not in a run
- 6 ON (pulsed) at the end of a run
- 7 ON (pulsed) when a peak is detected

The following diagram illustrates each of the options (1–7). Wait 1 identifies the time before the start of the first collection window.



To use the programmable output, connect the leads from Output 2 (9-10 pair) to a contact-activated input of another device. Connect either lead to either input pin on the peripheral device.

Then specify which one of the seven options to use. Access the Technical menu by pressing EDIT then 2. You'll see:

GSIOC unit ID =
Next Quit

Scroll through the menu screens by pressing *Next*, until you see:

Out 2 on [1 of seven choices] Next No Quit

To select the displayed choice, press END or *Next*. If you want one of the other choices, repeatedly press *No* until the screen displays the choice you want. Then press END or *Next* to select that choice. To exit the Technical menu, press *Quit*.

#### **Examples:**

- To turn on the strip chart after the initial wait period, you would connect Output 2 to an input pin of the strip chart and select the Out 2 ON after Wait 1 option.
- To turn on a pump during a run, you would connect Output 2 to an input pin on the pump and select the Out 2 ON during run option.
- To automatically start a new run after the previous one completed, you could connect Output 2 to Input A of the fraction collector and select the Out 2 ON after end of run option.



#### **GSIOC** interface

The Gilson Serial Input/Output Channel (GSIOC) is a bidirectional communications link which connects a master module (e.g., computer) with as many as 32 slave devices (in this case, a fraction collector).

The master communicates with one slave at a time. Each slave is identified by a unique unit ID code, designated by a number between 0 and 63. You can find or reset the GSIOC ID number in the Technical menu (EDIT 2); see *Appendix C*.

Using GSIOC, you can monitor and control the activity of the fraction collector and other Gilson equipment from a Gilson controller.

**Appendix F** describes in detail the commands used to control your fraction collector from a master module.

#### **Power Connection**

After you've made all the necessary connections, you're ready to attach the power cord and turn the power on.

When you turn on the power switch, the dispense head and arm will travel the farthest X-Y distances and then return to the "home" position at the left rear of the X-Y matrix.

The fraction collector display will dim after 10 minutes if:

- you haven't pressed a key
- a run has not been in progress
- power has not been turned off and then on

To brighten the display, press any key on the keypad. The display will become lit, but the function associated with the pressed key will not be performed.

If you send a remote contact signal to the fraction collector to start or end a run, this will brighten the display **and** start or end the run. If you send a remote GSIOC signal to the fraction collector, the display will become lit **and** the function associated with the signal will be performed.





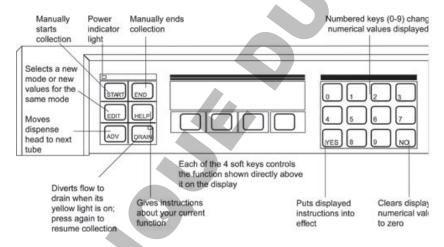
**Operation** 

3

The FC 203B Fraction Collector has a built-in front panel that you'll use to set collection mode and operating parameters. The 2-line display guides you through mode and parameter selection using a menu format. The keys provide full operating control.

#### This section describes:

- the front panel
- how to start the fraction collector
- how to review and change collection options
- how to indicate collection into the same set or different set of tubes for each collection cycle
- how to set collection options for each of the collection modes



## **Front Panel**

You give instructions to the fraction collector with three kinds of keys: command hard keys, numeric hard keys, and soft keys. You get information about the 203B software with the HELP key.

## **Command Hard Keys**

Hard keys are the labeled keys on the left side of the front panel. These keys put you in control of the fraction collector.

You can start or end a run, advance the dispense head, drain the effluent, and edit the run options with these keys. Use of the hard keys will be described in detail as necessary.

You'll recognize references to the hard keys because they're always written in CAPITAL letters.

## **Numeric Hard Keys**

Parameter values are entered on the numeric keypad at the right side of the fraction collector's front panel.

Also on this keypad are the NO and YES hard keys, used to change or confirm parameter values.

# **Soft Keys**

The four soft keys are located below the display. Their functions vary as you perform different operations.

It is the soft keys that make the fraction collector so easy to use. At all times, you'll know exactly which choices you can make. Simply press the soft key below the option you choose.

The examples in this manual illustrate the use of the soft keys. You'll recognize references to soft keys because they're always written in *italicized* letters.



Operation 3

# **Help Key**

The most useful hard key may be the HELP key. It's always available, to give you specific help and instructions every step of the way.

To acquaint yourself with the HELP instructions, press the HELP key any time during setup or operation.

This will not disturb any run in progress.



# **Start Up**

When all plumbing and electrical connections have been made, turn on the fraction collector. The arm and dispense head will start moving and the display will read:

Homing Collection Head FC 203B version X.XX

When the fraction collector "wakes up", it remembers and displays the last set of instructions it was given, for example:

Time mode 1.00 min/tube List Rack 0 Start

The fraction collector remembers:

- the last collection mode
- the most recent collection parameter values
- the last rack used

The fraction collector will automatically operate according to the "wake-up" instructions.

**Note**: To review the options that apply in the current mode, press the *List* soft key. If the options displayed are appropriate, simply prepare your vessels and press START. If the parameters need to be adjusted, read the next section, **Collection Options**.

**Note**: If a number greater than 0 was set for the *Tubes per cycle* parameter (see **Cycle Collection** section), a *Cycle* soft key appears instead of the *Start* soft key.

# **Collection Options**

# **Entering Numbers**

Numbers enter at the far right of the display and move to the left with each succeeding entry. The decimal point, where applicable, doesn't have to be entered.

One easy way to change an entry is to press the NO key to clear the current number. Then enter the new number just as you'd write it on a piece of paper.

For example, assume that two places are set to the right of the decimal point. To enter the number 68, clear the screen with the NO key. Then press "6". You will see:

0.06

Then press "8". You will see:

0.68

Finally, press "0" twice. You will see:

68.00

# **Review or Change Options**

To review the options that apply in the current mode, press the *List* soft key. Options and their current values are displayed one at a time.

If the displayed value is acceptable, press *Ok*. If you want to change a value, simply enter new numbers as described above.

## **Review or Change Racks**

To display a description of the current rack, press the *Rack* soft key. You'll see:

Rack code \_ selected [description of rack] Ok

To choose a different rack, enter the number code for the new rack. The code for most racks is imprinted on the rack.

From this display, press the HELP key to see a list of all rack codes in the 203B's memory.

Put the selected rack in place and press Ok.

# Collection Into Same or New Tubes for Each Cycle

You can have the fraction collector collect each sample into the same set of tubes or into a new set of tubes during each full collection cycle. If you collect into the same set of tubes, each cycle begins at tube 1. If you collect into a new set of tubes, the cycle begins at the tube following the last tube of the previous cycle.

To indicate which tubes are to be used during each cycle, press EDIT, then 2. Then press the *Next* soft key until you see:

Tubes per cycle = 0 Next Quit

If you want to collect each sample into the same set of tubes, indicate 0 for the tubes per cycle.

If you want to collect each sample into a different set of tubes, indicate a number greater than 0 for the tubes per cycle.

For more information, refer to the section called **Cycle Collection**.

# **Choosing Collection Modes**

To choose a collection mode, press the EDIT key. You'll see:

Select an edit path: Time Drop Peak Manl

Press the soft key corresponding to the mode of choice.

For detailed descriptions of the collection modes, refer to the section called **Collection Modes**.

# **Building Collection Windows**

To build collection windows, follow the detailed instructions in the section called **Collection Windows**.

## Ready to Start

**Note**: A change in the value of any parameter in any mode is immediately updated in every mode!

If you are not the only user of the fraction collector, check that the values you entered last have not been changed by someone else.

When the options, rack, and collection mode are acceptable and the display shows

\_\_\_ mode
List Rack \_\_Start

you're ready to start. Pressing either the *Start* soft key or the START key will start the collection.

**Note**: If a number greater than 0 was set for the Tubes per cycle parameter (see **Cycle Collection** section), a *Cycle x* soft key appears instead of the *Start* soft key. The x identifies which cycle will be started. To increment the cycle number, press the *Cycle x* soft key.

To start the run, press the START key at the left of the display. When the run finishes, the cycle number is incremented by 1. If the maximum cycle number was just completed, then the cycle number is set to 1.

If you press the END key during the run, the run terminates and the cycle number is incremented by 1.

If you press the END key when the ready-to-run display is being shown, the cycle number is set to 1.

# **During the Run**

#### **Manual Override**

During any run, you can use the ADV(ance), DRAIN, and END keys to override any automatic collection sequence.

When the ADV(ance) key is pressed, the dispense head immediately moves to the next vessel. During a run, the current vessel or fraction number is always displayed.

DRAIN is a two-state key. Press it once and effluent is diverted to the drain. Press it a second time and the 203B resumes collection in the current vessel. A yellow light on the DRAIN key indicates that the 203B is draining.

While the 203B is draining, pressing ADV does not resume collection. Instead it advances the number of the current tube, which is always displayed during a run. When DRAIN is pressed a second time, the 203B collects into the tube shown on the display.

Pressing END immediately ends the collection and sends the dispense head to "home".

#### **Power Failure**

In the case of a power failure during a run, the 203B will return to "home" and the display will flash:

Warning: power failed while collecting. -Ok-

This warns you that all effluent may not have been collected.

Press Ok to confirm the message. The dispense head returns to the "home" position. When the dispense head returns "home", it will not move directly over the fractions that have already been collected.

# **Cycle Collection**

The fraction collector enables you to collect fractions two different ways:

- Collect each sample, or full collection cycle, into the same set of tubes. During the run, each cycle begins at tube 1. This collection type is mostly used in preparative HPLC for collecting purified fractions.
- Collect each sample, or full collection cycle, into a different set of tubes. During the run, each cycle begins at the tube following the last tube of the previous cycle. This collection type is mostly used in analytical HPLC.

**Note**: For automated cycle collection, you must send a remote start signal to the 203B to begin each cycle. See page 2-15 for information on making the contact connection for remote start.

The *Tubes per cycle* parameter in the Technical menu enables you to indicate which way you want fractions collected. Using this parameter, you can divide the rack into sections with a set number of tubes per cycle. To display this parameter:

- Press EDIT, then 2.
- Press the *Next* soft key until you see:

Tubes per cycle = 0 Next Quit

#### **Collect into Same Set Tubes**

To collect each sample into the same set of tubes, set the *Tubes per cycle* parameter to 0.

*Example*. If you set 0 as the number of tubes per cycle for a Code 0 rack, each sample will be collected into the same set of tubes per collection cycle. Collection will be made into the following tubes for each cycle.

Cycle	Tubes
1	1-80
2	1-80
3	1-80
4	1-80
etc.	etc.

## **Collect into New Set Tubes**

To collect each sample into a new set of tubes, use the fraction collector's numeric keypad to indicate a number greater than 0 for the *Tubes per cycle* parameter. Using the tubes per cycle and the total tube capacity of the rack, the 203B calculates the number of cycles.

If the number of tubes can't be divided into the tube capacity evenly, the number of cycles is the whole-number quotient; the remaining tubes are not used.

**Example 1.** If you set 20 as the number of tubes per cycle for a Code 0 rack, the number of cycles would be 4. Collection will be made into the following tubes for each cycle.

$C_{\mathcal{I}}$	vcle	Tubes
1		1-20
2		21-40
3		41-60
4		61-80

**Example 2.** If you set 25 as the number of tubes per cycle for a Code 0 rack, the number of cycles would be 3. Collection will be made into the following tubes for each cycle.

Cycle	Tubes
1	1-25
2	26-50
3	51-75

## Ready-to-Run Display

When you set the tubes per cycle option, the *Start* soft key changes to a *Cycle x* soft key on the ready-to-run display. The *x* identifies which cycle will be started. To increment the cycle number, press the *Cycle x* soft key. By pressing the *Cycle x* soft key again after the maximum cycle number, *Cycle 1* can be redisplayed.

To start the run, press the START key at the left of the display. When the run finishes, the cycle number is incremented by 1. If the maximum cycle number was just completed, then the cycle number is reset to 1.

If you press the END key during the run, the run terminates and the cycle number is incremented by 1.

If you press the END key when the ready-to-run display is being shown, the cycle number is reset to 1.

**Note**: The tubes per cycle option cannot be used in conjunction with the multiple collection head option.

If you try to set the multiple collection head option when the tubes per cycle option is active, the 203B prompts you to change the *Tubes per cycle* parameter to 0.

If you try to set the tubes per cycle option when the multiple collection head option is active, the 203B prompts you to change the *Multiple collect heads* parameter to single.

## **Collection Modes**

The fraction collector can collect fractions in several ways.

#### Time mode

You can specify the collection time per fraction, in hundredths of minutes.

#### **Drop mode**

You can specify the number of drops collected per fraction.

#### Peak modes

If the fraction collector is connected to a detector, you can instruct the 203B to collect only peaks. A peak can be collected into many tubes. You decide whether you want to subdivide a peak by drops or time (minutes).

- peak + drop mode: You can specify the number of drops dispensed in each tube.
- peak + time mode: You can specify the time per tube.

In peak modes, you can also tell the 203B to collect non-peak effluent by time or by drops.

#### Manual mode

You can collect fractions manually, giving you complete freedom to advance the dispense head, drain the effluent, or end the run.

Collection modes are set by pressing the EDIT key. The next sections describe in detail how to set each collection mode.

# **Choosing Time Collection Mode**

#### **Mode selection**

Press the EDIT key.

You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press Time.

#### Time per tube selection

You'll see the time selection display:

Time mode \_\_ min/tube
Ok

Press the NO key to clear the display. Then enter the desired time per tube from 0.01 to 99.99 minutes.

Press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: Start to End
Ok Add
(If no windows have been built)

or

Collect: X.X to X.X.

Ok Add Del

(If windows have already been built)

**Note**: Collection windows let you specify time intervals during which effluent is collected or discarded. Refer to the section called **Collection Windows** to build, add, or delete collection windows.

Press Ok. Continue to press Ok if multiple collection windows have been set up.

## Ready-to-run display

You'll see the time mode ready-to-run display:

Time mode \_\_ min/tube List Rack \_\_ Start

From this display, you can:

- review or change list of options (Press *List*)
- review or change current rack (Press *Rack*)
- start a collection run (Press *Start*)
- change the min/tube value (Enter the new number)

**Note**: If Time had been the last mode selected, the 203B would wake up to this display.

**Note**: If a number greater than 0 was set for the *Tubes per cycle* parameter (see **Cycle Collection** section), a *Cycle x* soft key appears instead of the *Start* soft key. The *x* identifies which cycle will be started. To increment the cycle number, press the *Cycle x* soft key.

To start the run, press the START key at the left of the display. When the run finishes, the cycle number is incremented by 1. If the maximum cycle number was just completed, then the cycle number is reset to 1.

If you press the END key during the run, the run terminates and the cycle number is incremented by 1.

If you press the END key when the ready-to-run display is being shown, the cycle number is reset to 1.

# **Choosing Drop Collection Mode**

#### **Mode selection**

Press the EDIT key.

You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press Drop.

#### Drop per tube selection

You'll see the drop selection display:

Drop mode \_\_ drop/tube
Ok

Press the NO key to clear the display. Then enter the desired drops per tube from 1 to 9999 drops.

Press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: Start to End

Ok Add

(If no windows have been built)

or

Collect: X.X to X.X.

Ok Add Del

(If windows have already been built)

**Note**: Collection windows let you specify time intervals during which effluent is collected or discarded. Refer to the section called **Collection Windows** to build, add, or delete collection windows.

Press Ok. Continue to press Ok if multiple collection windows have been set up.

## Ready-to-run display

You'll see the drop mode ready-to-run display:

Drop mode \_\_ drop/tube List Rack \_\_ Start

From this display, you can:

- review or change list of options (Press *List*)
- review or change current rack (Press *Rack*)
- start a collection run (Press *Start*)
- change the drop/tube value (Enter the new number)

**Note**: If Drop had been the last mode selected, the 203B would wake up to this display.

**Note**: If a number greater than 0 was set for the Tubes per cycle parameter (see **Cycle Collection** section), a *Cycle x* soft key appears instead of the *Start* soft key. The x identifies which cycle will be started. To increment the cycle number, press the *Cycle x* soft key.

To start the run, press the START key at the left of the display. When the run finishes, the cycle number is incremented by 1. If the maximum cycle number was just completed, then the cycle number is reset to 1.

If you press the END key during the run, the run terminates and the cycle number is incremented by 1.

If you press the END key when the ready-to-run display is being shown, the cycle number is reset to 1.

# **Choosing Peak + Time Collection Mode**

Gilson strongly recommends using a 3-way valve when operating the 203B in this collection mode. Refer to *Section 2* for instructions on installing a 3-way valve.

#### **Mode selection**

Press the EDIT key.

You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press Peak.

#### **Fractionation selection**

You'll see:

Subfractionate peaks by: Time Drop Quit

To fractionate each peak into several tubes on the basis of time per tube, press *Time*.

## Time per tube selection

You'll see the time per tube selection display:

Peak mode \_\_min/tube Ok

Press the NO key to clear the display. Then enter the desired time per tube from 0.01 to 99.99 minutes.

If you want to collect each peak into a single tube, set a high value that will not overflow the tube (e.g., 30.00 min/tube).

Press Ok.



## Non-peak collection option

You'll see either of two displays:

or

Nonpeak: discard (time)
Ok Collect
(If non-peaks will be discarded)

You can choose whether to discard or to collect the effluent between peaks by toggling the *Discard/ Collect* soft key. The current selection is noted on the top line of the display.

If you choose to collect non-peak effluent, enter the non-peak collection time per tube using the numeric keypad.

When finished, press Ok.

#### **Setting peak parameters**

Before you can perform a peak collection, you must run a preliminary sample to collect information that helps the fraction collector determine when and where to look for peaks.

This involves a little extra effort from you, but that effort will be offset by improved results. You'll collect purer peaks and even collect overlapping peaks into separate tubes.

## Peak width display

The peak width is measured at 1/2 of a peak's maximum height. Measure the peak width on a peak of average width. This will ensure that narrower and wider peaks will also be collected. Use the X-axis scale to measure average peak width.

The peak width display looks like this:

Peak width: \_\_ min Ok

Enter the value in minutes. Then press Ok.

## Peak height display or peak level display

If the Collect by slope option is selected in the Technical menu, you'll be prompted for the minimum height of peaks to collect. Peak height enables the 203B to distinguish peaks from noise. From the millivolt (bottom to top) scale, determine the height (in millivolts) of the smallest peak you wish to collect. Use the Y-axis scale to measure minimum peak height.

The peak height display looks like this:

Peak height: \_\_ mV Ok

Enter the value in millivolts (mV). Then press Ok.

If you selected the Collect peaks by level option in the Technical menu, the display prompts you for a millivolt (mV) level:

Peak level: \_\_ mV Ok

Peaks above the indicated mV value will be collected while peaks below this value will be discarded. Use the Y-axis scale to identify the absolute threshold level.

If you have a relatively stable baseline, set the level value at about 2 times the expected baseline fluctuation. For example, if the baseline stays between 0 and 1 mV, set the level at 2 mV.

Appendix C describes the options in the Technical menu. Appendix H contains more information on peak detection by slope or mV level.

## **Delay time**

You'll see:

Delay time: \_\_ min
Ok

Delay time is the time required for liquid to move from the flow cell of the detector to the dispense head of the 203B. Tube advancement will be delayed by this amount of time, optimizing the collection of pure peaks. Delay time is more critical in analytical HPLC than in large-scale prep LC.

To calculate delay time, you'll need to know:

- flow rate (mL/min.)
- length of detector-to-collector tubing (cm)
- volume of inlet tubing from detector (μL/in.)

Find the volume of commonly used tubing in the following table:

Internal	<b>Diameter</b>	Volume
in.	mm	μL/in.
0.010	0.25	1.29
0.020	0.50	5.15
0.030	0.75	11.58
0.040	1.00	20.59

You can calculate delay time (minutes) with this formula:

Delay time = (length x volume)/flow rate

After entering the delay time value, press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: Start to End
Ok Add
(If no windows have been built)

or

Collect: X.X to X.X.

Ok Add Del

(If windows have already been built)

Note: Collection windows let you specify time intervals during which effluent is collected or discarded. Refer to the section called **Collection Windows** to build, add, or delete collection windows.

Press Ok. Continue to press Ok if multiple collection windows have been set up.

## Ready-to-run display

You'll see the peak + time mode ready-to-run display:

Peak mode \_\_ min/tube List Rack \_\_ Start

From this display, you can:

- review or change list of options (Press *List*)
- review or change current rack (Press *Rack*)
- start a collection run (Press *Start*)
- change the min/tube value (Enter the new number)

**Note**: If peak + time had been the last mode selected, the fraction collector would wake up to this display.

**Note**: The 203B has been preset to detect positive peaks. If your application requires detection of both positive and negative peaks, you may select that option via the Technical menu. See *Appendix C* for information.

**Note**: If a number greater than 0 was set for the Tubes per cycle parameter (see **Cycle Collection** section), a *Cycle x* soft key appears instead of the *Start* soft key. The x identifies which cycle will be started. To increment the cycle number, press the *Cycle x* soft key.

To start the run, press the START key at the left of the display. When the run finishes, the cycle number is incremented by 1. If the maximum cycle number was just completed, then the cycle number is reset to 1.

If you press the END key during the run, the run terminates and the cycle number is incremented by 1.

If you press the END key when the ready-to-run display is being shown, the cycle number is reset to 1.

# **Choosing Peak + Drop Collection Mode**

Gilson strongly recommends using a 3-way valve when operating the fraction collector in this collection mode. Refer to *Section 2* for instructions on installing a 3-way valve.

#### **Mode selection**

Press the EDIT key.

You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press Peak.

#### **Fractionation selection**

You'll see:

Subfractionate peaks by: Time Drop Quit

To fractionate each peak into several tubes, on the basis of number of drops per tube, press *Drop*.

## **Drop per tube selection**

You'll see the drop per tube selection display:

Peak mode \_\_\_ drop/tube Ok

Press the NO key to clear the display. Then enter the desired drops per tube from 1 to 9999 drops.

If you want to collect each peak into a single tube, set a higher value than the number of drops contained in a peak.

Press Ok.



## Non-peak collection option

You'll see either of two displays:

Nonpeak: \_\_ drop/tube
Ok Discard
(To collect non-peak fractions)

or

Nonpeak: discard (drop)

Ok Collect

(If non-peaks will be discarded)

You can choose whether to discard or to collect the effluent between peaks by toggling the Discard/Collect soft key. The current selection is noted on the top line of the display.

If you choose to *collect* non-peak effluent, enter the non-peak collection time per tube using the numeric keypad.

When finished, press Ok.

#### **Setting peak parameters**

Before you can perform a peak collection, you must run a preliminary sample to collect information that helps the fraction collector determine when and where to look for peaks.

This involves a little extra effort from you, but that effort will be offset by improved results. You'll collect purer peaks and even collect overlapping peaks into separate tubes.

## Peak width display

The peak width is measured at 1/2 of a peak's maximum height. Measure the peak width on a peak of average width. This will ensure that narrower and wider peaks will also be collected.

Use the X-axis scale to measure average peak width.

The peak width display looks like this:

Peak width: \_\_ min Ok

Enter the value in minutes. Then press Ok.

## Peak height display or peak level display

If the *Collect by slope* option is selected in the Technical menu, you'll be prompted for the minimum height of peaks to collect. Peak height enables the fraction collector to distinguish peaks from noise. From the millivolt (bottom to top) scale, determine the height (in millivolts) of the smallest peak you wish to collect. Use the Y-axis scale to measure minimum peak height.

The peak height display looks like this:

Peak height: \_\_ mV Ok

Enter the value in millivolts (mV). Then press Ok.

If you selected the Collect peaks by level option in the Technical menu, the display prompts you for a millivolt (mV) level:

Peak level: \_\_ mV Ok

Peaks above the indicated mV value will be collected while peaks below this value will be discarded. Use the Y-axis scale to identify the absolute threshold level.

If you have a relatively stable baseline, set the level value at about 2 times the expected baseline fluctuation. For example, if the baseline stays between 0 and 1 mV, set the level at 2 mV.

Appendix C describes the options in the Technical menu. Appendix H contains more information on peak detection by slope or mV level.

#### **Delay time**

You'll see:

Delay time: \_\_ min
Ok

Delay time is the time required for liquid to move from the flow cell of the detector to the dispense head of the 203B. Tube advancement will be delayed by this amount of time, optimizing the collection of pure peaks. Delay time is more critical in analytical HPLC than in large-scale prep LC.

To calculate delay time, you'll need to know:

- flow rate (mL/min.)
- length of detector-to-collector tubing (cm)
- volume of inlet tubing from detector (µL/in.)

Find the volume of commonly used tubing in the following table:

Internal	Diameter	Volume
in.	mm	μĹ/in.
0.010	0.25	1.29
0.020	0.50	5.15
0.030	0.75	11.58
0.040	1.00	20.59

You can calculate delay time (minutes) with this formula:

Delay time = (length x volume)/flow rate

After entering the delay time value, press Ok.

## **Collection window option**

You'll see the collection window display:

Collect: Start to End
Ok Add
(If no windows have been built)

or

Collect: X.X to X.X.

Ok Add Del

(If windows have already been built)

Note: Collection windows let you specify time intervals during which effluent is collected or discarded. Refer to the section called Collection Windows to build, add, or delete collection windows.

Press Ok. Continue to press Ok if multiple collection windows have been set up.

## Ready-to-run display

You'll see the peak + drop mode ready-to-run display:

Peak mode \_\_ drop/tube List Rack \_\_ Start

From this display, you can:

- review or change list of options (Press *List*)
- review or change current rack (Press Rack)
- start a collection run (Press Start)
- change the drop/tube value (Enter the new number).

**Note**: If peak + drop had been the last mode selected, the 203B would wake up to this display.

**Note**: The 203B has been preset to detect positive peaks. If your application requires detection of both positive and negative peaks, you may select that option via the Technical menu. See *Appendix C* for information.

**Note**: If a number greater than 0 was set for the Tubes per cycle parameter (see **Cycle Collection** section), a *Cycle x* soft key appears instead of the *Start* soft key. The x identifies which cycle will be started. To increment the cycle number, press the *Cycle x* soft key.

To start the run, press the START key at the left of the display. When the run finishes, the cycle number is incremented by 1. If the maximum cycle number was just completed, then the cycle number is reset to 1.

If you press the END key during the run, the run terminates and the cycle number is incremented by 1.

If you press the END key when the ready-to-run display is being shown, the cycle number is reset to 1.

# **Choosing Manual Collection Mode**

#### **Mode selection**

Press the EDIT key.

You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press Manl.

## **Delay time option**

You'll see the delay time display:

Delay time:\_\_\_\_ min
Ok

Delay time is the time required for liquid to move from the flow cell of the detector to the dispense head of the 203B. Tube advancement will be delayed by this amount of time, optimizing the collection of pure peaks. Delay time is more critical in analytical HPLC than in large-scale prep LC.

If needed, calculate the delay time as described for the peak modes. Press the NO key to clear the display. Then enter the desired delay time.

Press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: Start to End
Ok Add
(If no windows have been built)

or

Collect: X.X to X.X.

Ok Add Del

(If windows have already been built)

Note: Collection windows let you specify time intervals during which effluent is collected or discarded. Refer to the section called Collection Windows to build, add, or delete collection windows.

Press Ok. Continue to press Ok if multiple collection windows have been set up.

## Ready-to-run display

You'll see the manual mode ready-to-run display:

Manual mode
List Rack \_\_ Start

From this display, you can:

- review or change list of options (Press *List*)
- review or change current rack (Press Rack)
- manually start and stop the run

**Note**: If Manual had been the last mode selected, the fraction collector would wake up to this display.

## **Delayed advance function**

The manual mode of the fraction collector provides a special delayed advance function.

During a run in manual mode, you'll see:

Manual mode Delayed Tube Adv

Pressing the *Delayed Adv* soft key delays tube advance according to the delay time value entered.

The ADV key, on the other hand, causes an instantaneous advance.

The manual mode of the fraction collector can operate semiautomatically, if you take advantage of the collection windows and delayed advance capabilities.

However, you always have complete manual control in this mode. Just press START, ADV, DRAIN or END at the appropriate times. Make sure you understand the ADV/DRAIN interplay, described on page 3-9, before doing so.

## **Collection Windows**

Gilson strongly recommends using a 3-way valve when operating the fraction collector in this collection mode. Refer to *Section 2* for instructions on installing a 3-way valve.

Many users ask a fraction collector to collect a certain number of drops per tube or to collect each fraction for a defined length of time. Most of these users want to save every fraction, that is, to collect from start to end.

On the other hand, some users may know that during a particular time interval, no fractions of interest will elute from their column.

With the FC 203B Fraction Collector, you can define time intervals during which the effluent is either collected in fractions or is discarded. These time intervals are called collection windows.

When the fraction collector is **inside** a collection window, fractions are collected.

When the fraction collector is **outside** a collection window, the dispense head will move over the drain trough and divert the effluent to waste. When using a 3-way valve, the effluent will be diverted to waste via the valve.

#### Windows are universal

Collection windows apply in all modes: time, drop, peak + time, peak + drop, and manual.

If you select a new collection mode, the windows built in a previous mode remain active in the new mode. If you don't want to use those windows in the new mode, delete them.



#### **Examples**

#### Example 1. Start to end collection window

If you collect from start to end, your entire run is one long collection window, starting at time 0 and ending when the last fraction is collected.

#### Example 2. One open-ended window

If you know that nothing of interest will elute within the first 5 minutes of a run, you could instruct the fraction collector to drain the column effluent for 5 minutes before collecting fractions.

#### Example 3. Two windows

If you are collecting fractions for 0.5 minutes per tube and want to collect a peak which elutes at 4 minutes and another which elutes at 15 minutes, you could instruct the fraction collector to:

windows

drain for 3 minutes

collect 0.5 minute fractions for 2 minutes

(3–5 min.)

drain for 9 minutes

collect 0.5 minute fractions for 2 minutes

(14-16 min.)

#### Example 4. Two windows

If you are collecting fractions that contain 25 drops per tube and want to collect a peak which elutes at 4 minutes and another which elutes at 15 minutes, you could instruct the fraction collector to:

windows

drain for 3 minutes

collect 25-drops-per-tube fractions for 2 minutes (3–5 min.)

drain for 9 minutes

collect 25-drops-per-tube fractions for 2 minutes (14–16 min.)

**Note**: You may have noticed from Examples 3 and 4 that a collection window specifies a time interval, in any automatic mode, during which you can collect fractions either by length of time or by drop number.

The next section describes how to build windows to suit your collection needs.



## **Collection window display**

The collection window display looks like this:

Collect: Start to End

Ok Add

Start refers to 0 minutes, the beginning of each run. End refers to the last tube in a rack. It is represented by 999.9 min.

If you want to collect every fraction from *Start* to *End*, just press the *Ok* soft key.

## **Building One Window**

If you want to collect only a portion of the total effluent, build a collection window to specify when you want to collect.

The most common situation that requires a collection window is a single wait period at the beginning of a run. Suppose you want to discard the effluent for the first 2 minutes of a run.

**Note**: The time resolution when building collection windows is 0.1 minute.

## **Collection window display**

The collection window display will read:

Collect: Start to End

Ok Add

To add a collection window, press Add. You'll see:

Collect from: 0.0

Ok

Press 2, then 0 (Remember the fixed decimal point!). Then press *Ok.* You'll see:

Collect: 2.0 to 999.9

Ok



The End of a run is represented by 999.9 min.

Press Ok. You'll see:

Collect: 2.0 to End Ok Add Del

Press *Ok.* This window tells the 203B to discard the effluent for 2 minutes before it starts to collect fractions.

### **Building More Than One Window**

You can build up to 10 collection windows into a run.

Suppose you want to:

- discard the effluent for the first 5 minutes of a run
- then collect fractions for the next 5 minutes
- then discard for 10 minutes
- then collect until the end of the run

### **Collection window display**

The collection window display will read:

Collect: Start to End

 $Ok \quad Add$ 

To build the first collection window, press *Add*. You'll see:

Collect from: 0.0

Qk

Using this display, set the start time for the first collection window. Press 5, then 0 (Remember the fixed decimal point!). Then press *Ok.* You'll see:

Collect: 5.0 to 999.9

Ok

Using this display, set the end time for the first collection window. Press 1, 0, 0. Then press Ok. You'll see:

Collect: 5.0 to 10.0 Ok Add Del

This window tells the fraction collector to collect fractions between 5 and 10 minutes of the run.

To build the second collection window, press *Add*. You'll see:

Collect from: 0.0 Ok

**Note**: Your first collection window (5.0 to 10.0 minutes) has not been deleted! You'll always see 0.0 when you build a new window. Existing windows are not affected.

Press 2, 0, 0 (Remember the fixed decimal point!). Then press *Ok.* You'll see:

Collect: 20.0 to 999.9 Ok

Since you want to collect fractions until the end of the run (represented by 999.9 minutes), press Ok.

You'll see a review of the first window you built:

Collect: 5.0 to 10.0 Ok Add Del

Press Ok. You'll see the second window:

Collect: 20.0 to End Ok Add Del

Press Ok.

The review displays each collection window, organized from start to end of the run.

If you're building more than two collection windows, write them down first. You don't have to build in order, but doing so will simplify the process for you.

### **Deleting Windows**

If you don't want a window that is displayed, press *Del*.

To delete all collection windows, keep pressing *Del* until you see:

Collect: Start to End
Ok Add

### **Nonsense Windows**

The fraction collector won't let you build the two types of nonsense windows described below.

### Overlapped time

A window cannot start before another has ended. If you tried to add a third window to the previous example (e.g., collect from 25 to 30 minutes), you would see:

Overlapped time. Quit

To add the third collection window:

- press Quit
- press Ok until the Collect 20.0 to End window appears
- press Del to delete that window
- press Add and build the 25 to 30 minute window

### **Backward time**

The *end* time in a window must be later than the *start* time. If you tried to start collection at 2 minutes and end at 1 minute of the run, you would see:

Backward times. Quit

Press Quit and rebuild the window.



**Maintenance** 

4

The fraction collector is designed for reliable, long term use in normal laboratory and cold-room environments. Simple care and maintenance will ensure proper operation for many years.

### **Cold-Room Use**

You can leave the fraction collector in a cold-room, provided that the power is left on continuously. Leaving the power on provides enough internal heat to prevent condensation from forming on critical components.

### **Chemical Compatibility**

The case and keypad of the fraction collector are resistant to most solvents and buffers used in the lab. Follow good laboratory technique by cleaning spills with either water or alcohol.

You can easily remove the rack, pan, and drain trough for cleaning.

If you have questions concerning the chemical resistance of the 203B, contact your regional Gilson representative or call Gilson directly at 800-445-7661 or 608-836-1551. You can also contact Gilson Customer Service via its e-mail address: service@gilson.com

Outside the U.S., contact your Gilson representative

# **Transporting the Fraction Collector**

When moving the fraction collector to another location, do not use the arm as a handle. Doing so may damage the unit or cause misalignment of the X-Y positioning. You may carry the fraction collector using the large rectangular opening in the front of the unit.

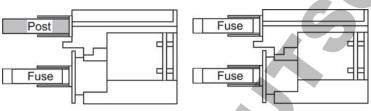
Fuse

drawer

### Replacing a Fuse

To change a fuse, follow these steps.

- 1 Disconnect the power cord from the power outlet and from the rear panel receptacle.
- 2 Locate the fuse drawer on the rear panel.
- 3 Insert a small screwdriver into the notch located to the right of the fuse drawer.
- 4 Twist the screwdriver to open and remove the fuse drawer. The fuse drawer contains one fuse for a 100/120 voltage selection. It contains two fuses for a 220/240 voltage selection.



Fuse drawer for 100/120 voltage selection

Fuse drawer for 220/240 voltage selection

5 Remove the old fuse(s) and insert the new fuse(s).

You must use "T" type fuses. The fuses that you install depend on the voltage of your unit.

Voltage selection	Fuse value	Fuse size
100 / 120V	0.5 amp	5 mm x 20 mm
220/240V	0.25 amp	5 mm x 20 mm

6 Insert the fuse drawer into its receptacle in the fraction collector.

A blown fuse may indicate the existence of another problem in the instrument. If the replacement fuses blow, don't try others. Contact your local representative or Gilson. See the **Before calling us** section on page 5-7.



Troubleshooting 5

If you encounter a problem while operating the FC 203B Fraction Collector, refer to the following pages. If you cannot solve or isolate the problem, contact the Gilson Customer Service Department or your local Gilson representative. See **Before calling us** on page 5-7.

### **Troubleshooting**

#### Machine makes loud noise

Connect the fraction collector to a grounded outlet.

The stepper motor and microprocessors in the fraction collector must establish a reference point ("home" position) each time the unit is turned on. "Home" position is at the left rear.

It is normal for the fraction collector to make a vibrating noise for a few seconds while the motors are "homing." This should occur only after the unit is turned on or when power is restored after a power failure. Minimize the noise by manually pushing the XY arm to the far left and the dispense head to the rear before turning the unit on.

## Unit makes loud noise when changing X or Y position in run

Check for obstruction to X or Y movement by turning off the fraction collector and moving the dispense head and XY arm. They should move smoothly.

### Drops are missing the vessel

Check to be sure rack code in program matches rack you are using.

Check rack location. Rack may be out of position on tray or locator base.

Check for a crimp in effluent tubing. Replace tubing into dispense head.

Check that tubing in the dispense head's drop detector is not bent.

Check mechanical XY arm assembly for alignment. Turn the unit off and then restart it. If still out of alignment, contact your local representative or the Gilson Customer Service Department.

## Drop detector not counting drops, or unreliable counting

Check that tubing in the dispense head's drop detector is not bent.

Check that tubing in the dispense head's drop detector is properly gauged. See page E-2.

Be sure the inlet tubing fitting is completely tightened so the tubing cannot move up and down.

Clean glass collar in drop detector (accessible from underside of dispense head; you will have to remove test tube rack). Use a cotton swab moistened with alcohol or water.

Replace glass collar in drop detector if unable to clean existing collar. Remove two small screws from underside of dispense head. Lift top piece off bottom of dispense head. Lift out glass collar and install replacement. Be careful not to break the wires connected to the dispense head.

Replace drop detector assembly (part number 170350V) if faulty. Instructions on how to replace this assembly are supplied with the replacement.

Replace electrical chassis.



### Separation problems in peak mode

Check peak parameters entered in the software. When collecting peaks by slope, peak height should be mV height of smallest peak. When collecting peaks by level, peak level should be mV value above which to collect peaks. Peak width should be average width of peaks at half height in chromatogram.

Specify negative peaks in the Technical menu (press EDIT 2 on keypad).

Event mark on UV trace interfering with peak detection. Connect event mark to recorder or data system rather than to event input of detector.

Bubbles in mobile phase creating spikes on recorder trace; degas mobile phase or eliminate plumbing leak that is causing bubbles.

Check that delay time (time for peak material to travel from detector to fraction collector) is set correctly.

### Not recognizing peaks in peak mode

Check detector input voltage. Should be less than 100 mV full scale.

Check peak parameters.

Check polarity. Connections from detector output to fraction collector input may be incorrect.

## 3-way valve not functioning or functioning incorrectly

Check connection to 3-pin socket of transition printed circuit board, accessible through XY arm opening. Plugging or unplugging valve with power on is not suggested as this may cause machine to reset.

Check that connector is oriented properly.

Check fluid connections to valve for proper orientation.

Contact your local Gilson representative or the Gilson Customer Service Department. Valve may be defective.

### Input functions not operating

Make sure connections into terminal block connector are secure.

Make sure terminal block connector is secure in multipurpose I/O port.

Check connections for proper pin assignments.

Be sure pins from external devices are assigned correctly.

Check polarity of input. Inputs should be a contact closure. If not, it must be TTL level (logic 0 activates).

Confirm that source supplying input to the fraction collector is working.

### **Output functions not operating**

Make sure connections into terminal block connector are secure.

Make sure terminal block connector is secure in multipurpose I/O port.

Check connections for proper pin assignments.

Output from the fraction collector should be compatible with the device to which it is interfaced. Outputs are contact closures.

**Note**: Output 2 mode is programmable in the Technical menu (press EDIT 2 on keypad).

### Unit not operational

Make sure power is turned on.

Check AC power cord connections.

Try different AC outlet.

Check fuse(s); replace if necessary.

Replace electrical chassis. Contact your local representative or the Gilson Customer Service Department.

#### **Unit blows fuses**

Contact your local representative or the Gilson Customer Service Department.

### **Repair and Return Policies**

### Before calling us

Gilson Customer Service personnel will be able to serve you more efficiently if you have the following information:

- serial number and model number of the devices involved. The serial number is on the back face of the 203B under the pan.
- installation procedure you used
- list of concise symptoms
- list of operating procedures and conditions you were using when the problem arose
- list of other devices connected to the 203B and a description of those connections
- list of other electrical connections in the room

### **Warranty repair**

Units covered under warranty will be repaired and returned to you at no charge. If you have any questions about applicability, please contact Gilson or your authorized representative.

### Non-warranty repair

For out-of-warranty repairs, contact your local Gilson representative or the Gilson factory. A Customer Service representative will discuss service options with you and can assist in making arrangements to return the equipment, if necessary.

### Rebuilt exchange

For some units, rebuilt exchange components are available. Contact Gilson for details.



### Return procedure

In the United States, contact the Gilson Customer Service Department to obtain authorization before returning any Gilson equipment. To return a piece of equipment:

- Carefully pack the unit to prevent damage in transit. Check with Gilson regarding proper method of shipment. No responsibility is assumed by Gilson for damage caused by improperly packaged instruments. Indicate the authorization on the carton and on the packing slip.
- Always insure for the replacement value of the unit.
- Include a description of symptoms, your name, address, phone number and purchase order to cover repair costs, return and shipping charges, if your institution requires it. Ship to:

Gilson, Inc. Attention: Customer Service (indicate the authorization here) 3000 W. Beltline Highway Middleton, WI 53562

Outside the United States, contact your Gilson representative for return procedures.

### **Racks**



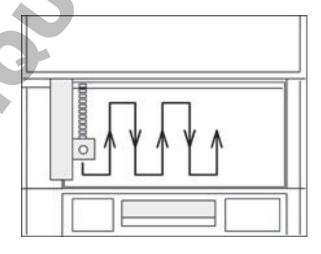
The FC 203B Fraction Collector accommodates many racks, all described in this appendix. Each rack has its own code number, and most of the racks have their code numbers imprinted on them.

The fraction collector always needs to know the code number for the rack you're using so it can accurately track the vessel positions for that rack. The diagram below shows the collection pattern used for almost all FC 203B racks. The only exceptions are:

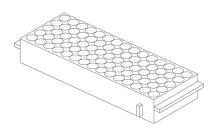
- Code 15 rack with microplate (see page A-5)
- Code 29 LE and 29 SE racks when used with capped Eppendorf vials (see pages A-7 and A-8)

Three racks (Code 30, 32, and 33) can maintain your fractions at a constant temperature. Tubes are placed in an aluminum block that is surrounded by a flowing water bath. To use these thermostated racks, you'll have to order:

- thermostating cuvette (part number 2704429)
- special rack holder for thermostated racks (part number 2704471). You will not need this rack holder when you use a Code 32 rack with 100 mm tubes.



**Collection Pattern** 



### **Collection plate**

For 80 vessels or direct collection into 80 cavities

Material: polypropylene

Cavity capacity: 2.5 mL/cavity

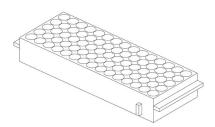
Vessels: 11 x 40 mm (1.5 mL) Eppendorf vials

without caps

Part number: 12240102, package of 20

Note: When setting up parameters, identify the

collection plate as rack code 0 (zero).



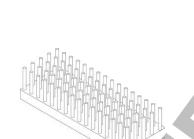
#### Code 0 rack

For 80 vessels

Material: polypropylene

Vessels and capacity: 12 x 32 mm vials (2 mL)

Part number: 270430



### Code 1 rack

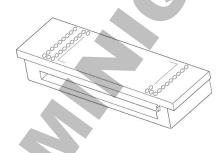
For 80 yessels

Material: polypropylene

Vessels and capacity: 13 x 65 mm tubes (7 mL)

13 x 100 mm tubes (9 mL)

Part number: 12240101, package of 6



### Code 2 rack

For 119 vessels

Material: polypropylene

Vessels and capacity: 6 x 50 mm tubes (1 mL)

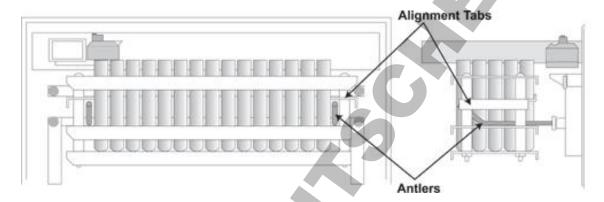
Part number: 130402

**Note**: Gilson recommends the use of a 3-way valve

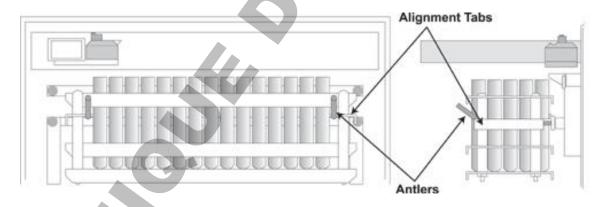
and small bore needle with this rack.

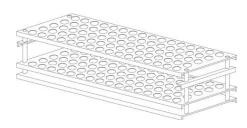
## Installation instructions for Code 4, Code 11 and Code 14 racks

- 1 Fill the rack with the appropriate collection tubes. (See page A-4.)
- 2 These racks each contain a pair of metal alignment tabs as illustrated below. Slide the rack onto the rack holder, keeping the alignment tabs ABOVE the antlers.



3 Firmly position the alignment tabs on the horizontal part of the antlers as shown below.





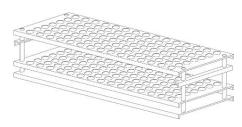
### Code 4 rack

For 80 vessels

Material: stainless steel

Vessels and capacity: 12 x 75 mm tubes (5 mL)

Part number: 130412 Installation: see page A-3



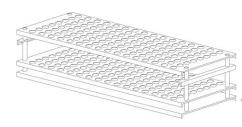
### Code 11 rack

For 105 vessels

Material: stainless steel

Vessels and capacity: 13 x 100 mm (9 mL)

Part number: 170413 Installation: see page A-3



### Code 14 rack

For 128 vessels

Material: stainless steel

Vessels and capacity: 12 x 75 mm tubes (5 mL)

Part number: 170414 Installation: see page A-3



### Code 15 rack holder

For a standard or deep-well microplate

Material: aluminum

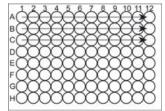
Vessel capacity: 0.3 mL/well

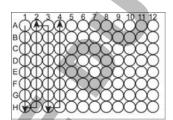
Part number: 170415

Installation: Do one of the following.

- If you are using a standard microplate, align the microplate holder with the upper set of holes on the front standoffs of the fraction collector. Attach the holder using thumbscrews. Place the microplate onto the holder.
- If you are using a deep-well microplate, align the microplate holder with the lower set of holes on the front standoffs of the fraction collector. Attach the holder using thumbscrews Place the microplate onto the holder.

Collection pattern: Refer to diagrams and their related text.





Indicate rack code "15" in the software if you want to collect from left to right Indicate rack code "17" in the software if you want to collect in a serpentine pattern

**Note**: A 3-way valve and small bore needle are required for collection into a microplate.





#### Code 20 rack

For 108 vessels

Material: polypropylene

Vessels and capacity: 10 x 100 mm tubes (4.5 mL)

Part number: 150425



### Code 21 rack

For 60 vessels

Material: polypropylene

Vessels and capacity: 13 x 100 mm tubes (9 mL)

Part number: 150422



### Code 23 rack

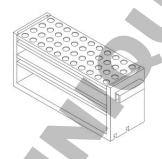
For 44 vessels

Material: polypropylene

Vessels and capacity: 17 x 55 mm vials (6.8 mL)

17 x 65 mm vials (8 mL)

Part number: 150426



### Code 23W rack

For 44 vessels

Material: polypropylene and stainless steel Vessels and capacity: Waters WISP vials (4 mL)

Part number: 270433

Note: When setting parameters in the software,

indicate rack code "23".

#### Code 24 rack

For 14 vessels

Material: polypropylene

Vessels and capacity: 28 x 60 mm (20 mL)

scintillation vials Part number: 150427



For 108 vessels

Material: polypropylene

Vessels and capacity: 10 x 65 mm tubes (3 mL)

10 x 75 mm tubes (4 mL)

Part number: 150420



For 60 vessels

Material: polypropylene

Vessels and capacity: 12 x 65 mm tubes (3.5 mL)

12 x 75 mm tubes (5 mL)

Part number: 150429

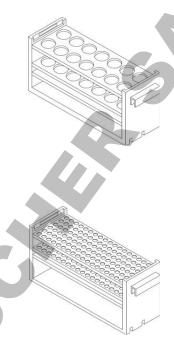
### Code 29 LE rack

For 60 large (1.5 mL) Eppendorf vials Material: polypropylene and stainless steel Vessels and capacity: 11 x 40 mm (1.5 mL)

Part number: 2704342

Note: When setting parameters in the software,

indicate rack code "29".









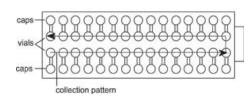
### Code 29 SE rack

For 60 small (0.5 mL) Eppendorf vials Material: polypropylene and stainless steel Vessels and capacity: 7 x 30 mm (0.5 mL)

Part number: 2704341

Note: When setting parameters in the software,

indicate rack code "29".



## Using capped Eppendorf vials in Code 29 LE or 29 SE rack

Place the vials in the center two rows with the caps facing outward. When setting parameters in the software, indicate rack code "19".



### **Thermostated Racks**

To use the thermostated racks described below, you'll have to order:

- thermostating cuvette (part number 2704429)
- special rack holder for thermostated racks (part number 2704471). You will not need this rack holder when you use a Code 32 rack with 100 mm tubes.

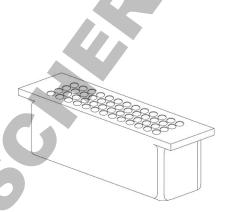
#### Code 30 rack

Thermostated rack for 60 vessels

Material: aluminum

Vessels and capacity: 12 x 32 mm vials (2 mL)

Part number: 2704430



#### Code 32 rack

Thermostated rack for 60 vessels

Material: aluminum

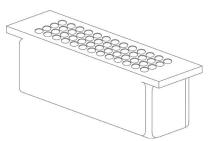
Vessels and capacity: 13 x 65 mm tubes (7 mL)

13 x 100 mm tubes (9 mL)

Part number: 2704432

Note: When using a Code 32 rack with 13 x 65 mm

tubes, set rack code to 30 in the software.



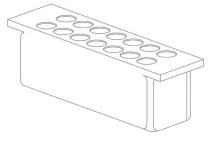
### Code 33 rack

Thermostated rack for 14 vessels

Material: aluminum

Vessels and capacity: 28 x 60 mm vials (20 mL)

Part number: 2704433





### **Parameter Review Menu**

B

When you store parameters within any fraction collection mode, those parameters are active in every mode. For example, a collection window established in drop mode is also active in time mode.

If your fraction collector has several users, you must take care to review all parameters before beginning a run.

The fraction collector provides a convenient list of parameters and current values for review. You can also change values from this menu. The new values you enter here will apply whenever that parameter applies.

To access this menu, press EDIT, then 1. You'll see, in order:

 $\begin{array}{ccc} \textit{Time mode} & \_\_ & \textit{min/tube} \\ \textit{Ok} & & \\ \end{array}$ 

 $\begin{array}{ccc} Drop & mode & \_ & drop/tube \\ Ok & & & \end{array}$ 

Peak mode \_\_ min/tube Ok

Peak mode \_\_ drop/tube Ok

Nonpeak: discard (time)
Ok Collect

Nonpeak: discard (drop) Ok Collect

or

Nonpeak: \_\_ drop/tube Ok Discard

Peak width: \_ min Ok

Peak height: \_\_ mV Ok

or

Peak level: \_\_ mV

Delay time: \_\_ min
Ok

Collect: Start to End

Ok Add

or

Collect: \_\_ to \_\_ Ok Add Del

Select an edit path:

Time Drop Peak Manl

From this final display, you can proceed into setup for operation in the mode of your choice.



## **Technical Menu**

parameter in this menu.

Access the Technical menu by pressing EDIT, then 2. Following is an explanation of each

GSIOC unit ID = 6 Next Quit

The unit ID identifies the 203B to a master device (or a computer) when the two are connected via the Gilson Serial Input/Output Channel (GSIOC). As a default, the unit ID is set to 6. There is no need to change this number unless it conflicts with another unit's ID code. For example, if you had two 203Bs connected along the GSIOC, assign one of them a different unit ID number.

Each ID number must identify a unique device. For communication to occur, the 203B and the computer must "agree" on the same unit ID number. Use the numeric keypad to change the unit ID.

GSIOC baud rate []
Next Quit

If the fraction collector is connected to a master device, use this parameter to set the baud rate for data transmission between the two devices.

For most Gilson systems, the rate of transmission is clocked outside of the device. Therefore, choose external baud by pressing 0 on the keypad. For example, if you connected the 203B to a Gilson 506C System Interface, make sure external baud is selected.

When the master device does not provide a clock, change the baud rate to the value the master device is currently using. Press 3 for 300, 6 for 600, 12 for 1200, 24 for 2400, 48 for 4800, 96 for 9600, and 192 for 19200.

Out 2 on [1 of seven choices] Next No Quit

During a run, you can have the fraction collector activate another device by connecting the programmable output 2 (9-10 pair) to the other device. To select the time point at which the fraction collector activates the other device, repeatedly press *No* until the screen displays the choice you want. Then press END or *Next* to select that choice. See **Wiring to Other Equipment** in *Section 2* for more information.

Collect positive peaks
Next Both Quit
or
Collect both peak types
Next Pos Quit

Press the *Both* soft key if you want the fraction collector to collect both positive and negative peaks during a run. Note that when the fraction collector is set to look for negative peaks, it is less able to accurately interpret difficult chromatograms. Press *Pos* if you want only positive peaks collected. *Appendix H* describes how the fraction collector locates peaks.

Single collection head Next Multi Quit or Multiple collect heads Next Single Quit

If you're collecting effluent from one column, set the Single collection head option. If you want to collect effluent from several columns at the same time, make sure the *Multiple collect head* option is selected. See *Appendix G* for more information about multiple collection heads.

Tubes per cycle = 0 Next Quit

The *Tubes per cycle* parameter enables you to divide the rack into sections with a set number of tubes per cycle. When collection begins, each cycle will be a full collection cycle, start to end. See **Cycle Collection** in *Section 3* for details on how to set this option.

Collect peaks by slope.

Next Level Quit

or

Collect peaks by level.

Next Slope Quit

The Collect peaks by parameter identifies the procedure that the fraction collector will use to collect peaks while in peak mode. (See Section 3 for information on how to set parameters in peak mode.)

If you select the slope option, the fraction collector collects peaks using a peak detection algorithm. To distinguish peaks from noise, the algorithm uses the height of the smallest peak of interest and the width of an average peak, measured at half height.

If you select the level option, the fraction collector uses a defined millivolt (mV) value along with the width of an average peak, measured at half height, for peak collection. Peaks above the specified mV level are collected while peaks below the level are directed to waste. Peak width is used to filter noise, to determine the width of a safety window so the software does not exit a peak prematurely, and to identify when the software can exit a peak.

See *Appendix H* for more details on peak detection by slope or mV level.

## **Service Menu**

D

This list of parameters is available to help service personnel confirm satisfactory instrument performance or to aid in troubleshooting.

Access the Service menu by pressing EDIT, then 3. For an explanation of a parameter in this menu, press the HELP key when the parameter is on the display.

The parameters in this menu are:

A/D slope: \_\_

Next Quit

Detector A/D:  $\mu V$ 

Next Zero Quit

 $Serial\ port = \_\_\ h$ 

Next Quit

Begin XY test run?

Next Test Quit

Erase all options?

Next Erase Quit

Align collection head?

Next Align Quit

Software Version 203Bv\_\_.\_

Next

# **Dispense Head End-Fitting Installation**



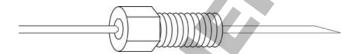
With time and use, the tip of the inlet tubing and the end fitting may become worn or damaged.

To ensure regular drop formation, you may have to reposition or replace the  $Omnifit^{TM}$ -type fitting or recut the end of the tubing.

### Replacement

To replace the end fitting, use the following instructions:

Pull the Omnifit<sup>TM</sup> gripper off the tube. Cut the tubing at a shallow angle with a razor blade, as shown below.

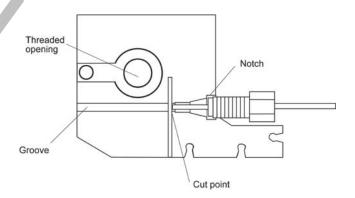


Replace the gripper so it rests about 3 cm from the tip. Use a needle nose pliers to pull the tubing through the gripper.

Cut off the angled portion of tubing so it looks like this:



The dispense head of the fraction collector contains a gauge to indicate the distance the tubing should extend beyond the gripper. Place the tubing assembly into the groove on the dispense head, as shown below.



With the gripper resting against the notch, cut the tubing off at the cut point. Now attach the tubing assembly to the dispense head.

## Repositioning

If you only need to reposition the gripper or recut the end, slide the gripper back on the tube and use the tubing gauge to set the proper length.



## **GSIOC** Interface



The Gilson Serial Input/Output Channel (GSIOC) is an asynchronous serial communications interface that enhances the power of your Gilson equipment.

It incorporates an EIA RS-485 interface and allows 32 slave devices to be controlled from a single master in a multi-drop configuration.

A device is identified by a unique number which must be known to the device and to the software. The default ID code of the FC 203B Fraction Collector is 6. You can set it to any number from 0 to 63, but remember to use the same value in the software when issuing commands to the fraction collector. Also make sure that no other unit in the system has the same ID number.

From the software, you:

- specify the device that you want to control
- issue commands that set operating parameters, control operation, or request information from the device

**Note**: For more information on the GSIOC interface and GSIOC commands, refer to the *Technical Manual* for the FC 203B Fraction Collector.

To control the fraction collector via the GSIOC interface, you will need the following equipment:

- A computer
- Gilson 506C System Interface or 605 RS-232 Adapter
- Gilson 712 HPLC Control Software, UniPoint™ System Software, or 706 Device Driver Software and your program

### **GSIOC Commands**

There are two kinds of commands that you can issue from your master unit to the fraction collector.

- Immediate commands request status information from the fraction collector. These one character, uppercase or symbol commands are executed immediately, temporarily interrupting other GSIOC commands in progress.
- Buffered commands send instructions to the fraction collector. These commands are executed one at a time.

#### **Immediate Commands**

#### Command Description

\* Reads the contact status of the fraction collector inputs. Returns "abcd" where:

a = C if input A is shorted (closed)D if input A is open

b = C if input B is shorted (closed)
D if input B is open

c and d always = D in the FC 203B

- ? Reads contact status of the fraction collector outputs. Returns "abcdef" where:
  - a = C if output 1 is closed D if output 1 is open
  - b = C if output 2 is closed D if output 2 is open

cdef always = D in the FC 203B

- \$ Resets the fraction collector. It is equivalent to restarting the unit.
- % This command reads the current version. Returns "203Bvx.x" where:

x.x = currently installed software version

- 0 Reads FIFO 0. Format is defined by data transfer protocol.
- 9 Reads event FIFO. Returns "Xtttttt" where:

X = @ if contact 1 open, contact 2 open

A if contact 1 closed, contact 2 open

B if contact 1 open, contact 2 closed

C if contact 1 closed, contact 2 closed

| if no event is available in queue

tttttt = the time (in 10 ms units) since the last buffered 9 command or 0000000 if the queue is empty

K	Reads	pressed	keys.	Returns	"k"	where
K	Reads	pressed	keys.	Returns	"K"	where

k = ASCII code for key or null (ASCII 0)

Reads both lines of the display. Returns "eline number 1> eline number 2> a' where:

line number 1> = the upper 24 characters

line number 2> = the lower 24 characters

a = + if drain is on- if drain is off

r Reads the top line of the display. Returns "line number 1>" where:

line number 1> = the upper 24 characters

T Reads current tube number. Returns "x.xx" where:

xxx = the current tube

000 indicates that the head is not over a defined tube.

V Reads raw analog input voltage. Returns ".xxxxxxV".

Resolution is 1 microvolt.

X Reads X-axis position. Returns "axxxx" where:

a = M if moving
S if stationary

xxxx is in 0.1 mm units

Y Reads the Y-axis position. Returns "axxxx" where:

a = M if movingS if stationary

xxxx is in 0.1 mm units

#### **Buffered Commands**

Command Description

Closes contact output

C1 closes output 1 C2 closes output 2 C12 closes both outputs

D12 Opens contact output

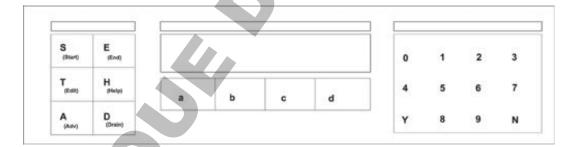
D1 opens output 1 D2 opens output 2 D12 opens both outputs

Gddd Causes the fraction collector to beep for a specified period of time:

**ddd** specifies beep duration (0 to 100); units are 0.1 seconds (for example, 100 = 10 seconds).

K<string>

Simulates front panel key strokes via GSIOC. You can enter up to a 40-character string. Keystrokes are simulated according to the key assignments shown below. For example, if you wanted to start a run, you would issue the command KS.



Ls Locks or unlocks the front panel:

s = 0 for lock front panel 1 for unlock front panel Mxy Relaxes motors.

Mx relaxes X motor.
My relaxes Y motor.
Mxy relaxes both motors.

Warning: After you use this command, reset the fraction collector using the immediate \$ command before you issue a command that moves the head to a new position.

Px Enables remote control of peak collection. For x, you can enter:

"p" to indicate a peak
"b" to indicate a baseline

"m" to show transition between merged peaks

"?" to have the fraction collector resume peak collection

Txxx Moves head to the specified tube. The xxx parameter indicates the number

of the tube.

Vx Flips 3-way (diverter) valve:

x = 1 for divert to drain0 for don't divert to drain

Wx<string> Writes text to the upper or lower line of the display:

x = 1 for the upper line 2 for the lower line

<string> can be up to 24 characters

Xxxxx Sets the X-axis position indicated by xxxx. Units are 0.1 mm towards the

right.

Yxxxx Sets the Y-axis position indicated by xxxx. Units are 0.1 mm towards the

front.

0xxxx Sets FIFO 0 sampling rate and clears FIFO. The parameter xxxx is the

sampling rate in units of 0.01 Hz.

9 Clears the contact closure event FIFO and resets the contact event timer.

## **Multiple Column Option**

G

The FC 203B Fraction Collector's multiple column option allows you to collect effluent from several columns at the same time.

You can use the multiple column option with any Gilson rack, in any operating mode. To easily synchronize collection from several columns, Gilson recommends that you choose Time mode as the operating mode.

Before you use this option, use the following instructions to:

- prepare your columns
- select the multiple column option
- install and adjust the multiple column adapter
- install a rack

#### Set-up

Prepare as many separation columns as you will need. Attach the column outlet tubings to the columns.

**Note**: Remember to make the outlet tubings long enough to move with the multiple column adapter during the run!

#### **Column Option Display**

To access the multiple column option from the Technical menu, press EDIT 2, then *Next* until you see:

Single collection head Next Multi Quit

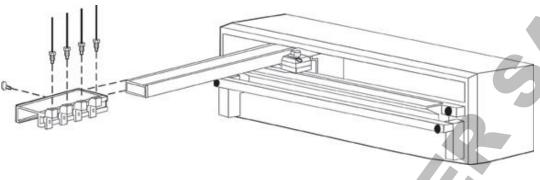
or

Multiple collect heads
Next Single Quit

- If the first line shows Single collection head, press Multi to tell the fraction collector that you'll run multiple columns. Press Quit to return to the operating mode you selected.
- If the first line shows Multiple collect heads, the fraction collector is already set to use multiple columns. Press Quit to return to the operating mode you selected.

When you choose the multiple column option, the motor that controls front to back movement of the dispense head is relaxed. During collection, the dispense arm will move only from left to right (when viewed from the front of the fraction collector).





#### **Install Column Adapter**

Push the drop detector head to the rear of the dispense arm.

With the nylon thumbscrew facing the left hand side of the fraction collector, slide the multiple column adapter onto the dispense arm.

- If you will collect in manual, time, or peak + time mode, push the multiple column adapter as far back as it will go. Tighten the thumbscrew.
- In drop mode and peak + drop mode only, you can key tube advance to the number of drops passing the drop detector. However, you will not be able to collect fractions in the row of tubes adjacent to the drop detector head.

If you choose to collect in a drop mode, position the drop detector over the tube closest to the rear of the fraction collector. Then push the multiple column adapter back until it touches the drop detector head. Tighten the thumbscrew.

#### **Connect Outlet Tubing**

Connect each column outlet to an adjustable tubing holder on the multiple column adapter.

Place an Upchurch flangeless nut and ferrule onto the end of each outlet tubing. Your accessory package contains nuts and ferrules for 1/16" and 1/8" tubing. Be sure the cone of the ferrule faces the nut.



Flangeless nut and ferrule for 1/16" tubing



Flangeless nut and ferrule for 1/8" tubing

Loosely screw the fitting into the top of the tubing holder. Place the Allen wrench into the set screw on the tubing holder.

Push the tubing through the fitting until it touches the Allen wrench. Then fingertighten the 1/4-28 fitting.

**Note**: If you will collect in a drop mode, as described above, you must attach one column outlet to the drop detector head. To make this connection, follow the **Plumbing Setup** instructions in **Section 2**.

#### **Install Rack**

Select and install a rack, considering these points:

- Fractions eluting from each separation column are collected in a single row from left to right across the length of each rack. You must allocate one row for each chromatographic column.
- Each row must contain enough vessels to collect the desired number of fractions.

**Example**: A Code 1 rack holds 80 tubes arranged in a 5 x 16 array. With this rack installed, you could collect up to 16 fractions from as many as five columns.

• If a microplate holder and microplate are installed, indicate rack code "17" to collect into the microplate.

#### **Align Outlet Tubings**

Carefully align the outlet tubing from each column so effluent will fall into a single row of tubes in the rack.

Using the Allen wrench, tighten each adjustable tubing holder when you are certain that it is properly aligned.

**Note**: The outlet tubing will not be directly over the tube until the run starts. You should ensure that the drops will fall into the tube by looking at the tubing holders from the right-hand side of the fraction collector.

### **Begin the Run**

Begin the collection by pressing START. Notice that instead of showing the current tube number, the display shows the number of the fraction being collected.

#### **End the Run**

After the final fraction has been collected, a short alarm will sound. At that time, you should follow this method to prevent extraneous material from dripping into the tubes:

- stop the flow from the columns! You must do this first.
- press END to finish the run. The dispense head will remain at the right-hand side of the rack until you press END.
- remove the rack of tubes.

**Note**: If you are using the programmable output (Out2) to control another device, be aware of the following change that is in effect when collecting from several columns.

If the output was scheduled to turn a device on or off at the end of a run, it will now do so after the last fraction has been collected.

## Return to Single Column Collection

When you want to return to single column collection, remove the multiple column adapter.

Then access the column option from the Technical menu (EDIT 2) and choose *Single*. The display will remind you that the dispense arm is being reset.

You can then proceed with single column collection.

# Peak Detection Using Slope or Millivolt Level



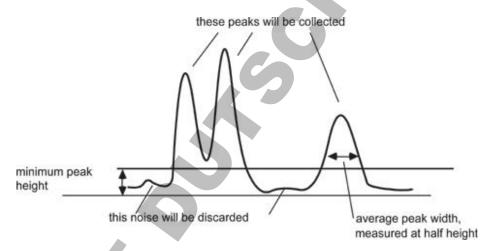
In the Technical menu (EDIT 2), you indicate if the fraction collector should collect peaks using slope or millivolt level. (Collection by slope is the default.) This appendix describes the peak detection algorithm that the fraction collector uses to collect peaks if the slope option is selected. It also looks at what peaks are collected if you choose the level option.

## **Detection Using Slope (Peak Detection Algorithm)**

To distinguish peaks from baseline noise, you tell the fraction collector the following information when setting parameters in the peak mode:

- · height of the smallest peak of interest
- width of an average peak, measured at half height

You can get the above information by running a preliminary sample.

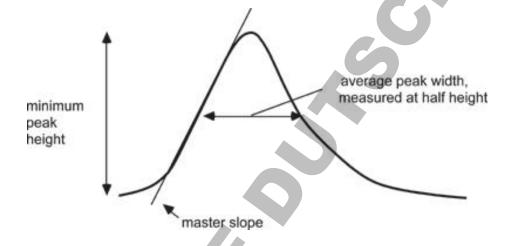


#### **Master Slope**

When you enter values for minimum peak height and for average peak width, the fraction collector determines the master slope of the smallest peak that it will detect.

Master slope  $(S_0) = (k_0)$  Peak height Peak width

As you increase the peak width setting and/or decrease the peak height setting, the master slope decreases and the peak-detection sensitivity increases.



#### **Peak Phases**

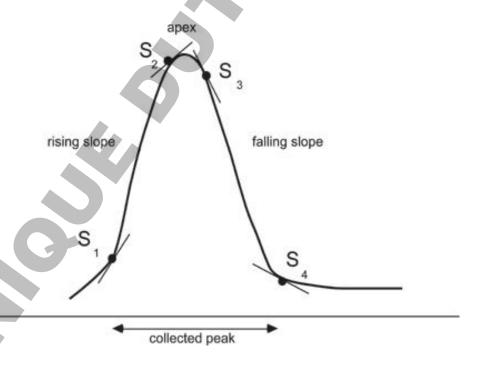
A positive peak is composed of three phases (refer to diagram below):

- a rising slope
- an apex
- a falling slope

The fraction collector uses a phase-specific slope detection method to determine the presence of a peak. Critical slopes at four points define the three peak phases and the return to baseline.

Those critical slopes are related to the master slope, S<sub>0</sub>:

rising slope  $(S_1)$  =  $k_1S_0$ apex slope  $(S_2)$  =  $k_2S_0$ falling slope  $(S_3)$  =  $k_3S_0$ baseline slope  $(S_4)$  =  $k_4S_0$ 



#### **Maximum Peak Width**

The maximum valid width of a peak is also determined from the master slope parameters (peak height and width):

Maximum width =  $k_5 S_0$ 

(k<sub>0</sub> through k<sub>5</sub> are programmed constants)

When the maximum peak width is exceeded, the fraction collector decides that a baseline shift has occurred and it resets the baseline appropriately.

#### **Special Cases**

If you desire, you can collect negative peaks. The three negative-peak phases are falling front, bottom, rising end. In general, when the fraction collector is set to look for negative peaks, it is less able to accurately interpret difficult chromatograms. The default setting is for collection of positive peaks only. To change that setting, see *Appendix C*.

The fraction collector can identify merged peaks, recognizing that the falling slope phase of one peak is directly adjacent to the rising slope phase of the next. Positive merged peaks and negative merged peaks can be identified.

## **Detection Using Millivolt Level**

To collect peaks using a millivolt (mV) level, you tell the fraction collector the following when setting parameters in the peak mode:

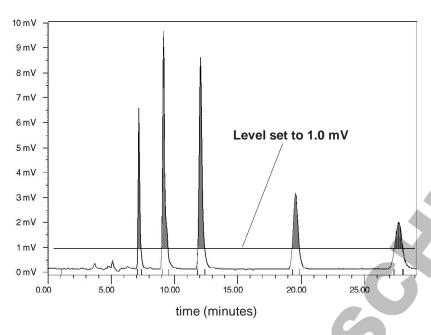
- width of an average peak, measured at half height
- mV value above which all peaks will be collected

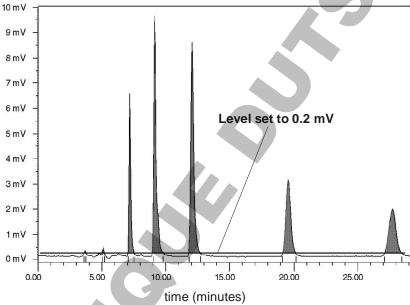
You can get the above information by running a preliminary sample.

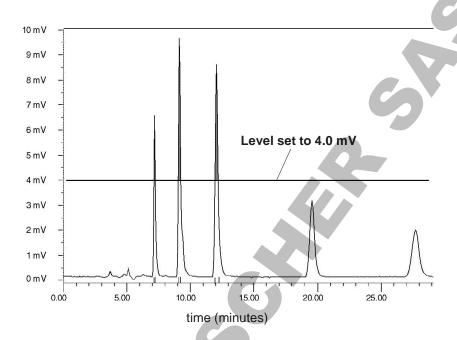
The fraction collector uses the mV value along with the peak width for peak collection. Peaks above the specified mV level are collected while peaks below the level are directed to waste. Peak width is used to filter noise, to determine the width of a safety window so the software does not exit a peak prematurely, and to identify when the software can exit a peak.

As a recommendation, set the mV level at 2 times the expected baseline fluctuation. For example, if the baseline stays between 0 and 1 mV during collection, set the level at 2 mV.

The shaded peak areas and event marks in the following chromatogram plots show how setting the level at different mV values affects the amount of effluent collected for peaks.







## **Autopreparative Collection Controlled** by 712 HPLC Control Software

5

In a Gilson autopreparative HPLC system, one high-pressure pump repetitively injects sample. The autopreparative system can be an isocratic or a gradient system and will include a fraction collector, such as the 203B.

To coordinate system components, you'll use Gilson 712 HPLC Control Software or UniPoint<sup>TM</sup> System Software. Within the software, you'll set parameters that control the devices via the Gilson Serial Input/Output Channel (GSIOC) or by sending contact signals.

This appendix describes how to set up system components for the autopreparative collection of fractions using the 712 HPLC Control Software. You'll find information on making rear panel connections, setting parameters on the fraction collector, and setting parameters in the 712 HPLC Control Software. In the examples, the injection pump has a 10 SC head, and the injection volume is 5 mL at a flow rate of 5 mL/min. Also four samples will be injected and eight fractions will be collected from each sample.

To learn about controlling an autopreparative system using UniPoint, please request a copy of the Large-Volume Preparative System Control Using a 30x Pump for Repetitive Injection Technical Note (part number LT21375) from your Gilson representative.

#### **Connecting System Components**

This section describes the electrical connections you need to make between the fraction collector, 506C System Interface, and a detector.

#### **GSIOC** Connections

To control the pumps and send GSIOC commands to other system components, use GSIOC cables to connect system components to the 506C System Interface. Then connect the RS-232 cable to the serial ports of the 506C and the computer.

If necessary, refer to the *System Installation* manual that came with the control software.

#### **Contact Connections**

Use 2-conductor cable to make contact connections between the fraction collector and the 506C.

To remotely start the fraction collector, send an output signal to the fraction collector from the computer via the 506C. Connect the fraction collector Input A to the 506C Output 1. Be sure to match ground connections. Page I-13 describes how to set up the event to send the output signal from the computer.

To mark tube advance directly on the data chart, connect the fraction collector event mark output (Output 1, 7-8 pair) to Input A of the 506C. Be sure to match ground connections.

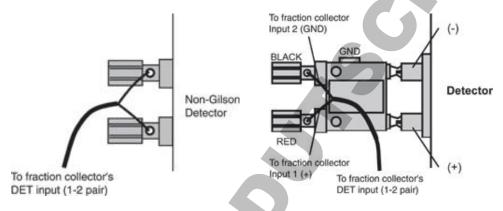
**Note**: If you're collecting in peak mode and also want an event mark at peak start, see page 2-17.



#### **Detector Connections**

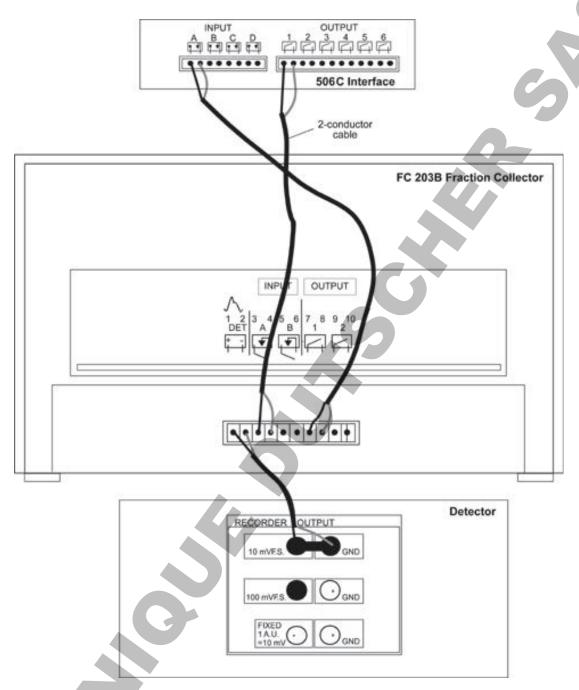
In addition, if collecting in peak mode, connect the detector to the fraction collector's DET input. (If you're using a Gilson detector, you'll need a Pomona cable adapter, part number 6374022611.) Connect the fraction collector's DET pin 1 to the detector's "+" output and the fraction collector's DET pin 2 to the detector's "-" (or GND) output.

**Note**: If you're using a non-Gilson detector, you may need to connect the DET pin 2 to the metal screw in the lower left or right corner of the fraction collector to reduce ground noise. Refer to page 2-14.



Connection for a non-Gilson detector

Connection for a Gilson detector



Contact and Detector Connections for 203B and 506C

### **Programming the 203B**

The fraction collector enables you to collect into the same set of tubes or into a different set of tubes in each cycle.

The following examples show how to set up parameters for a run using the time mode. One example collects each cycle into the same set of tubes while the other collects into a different set of tubes.

## Example 1 Collection of Each Cycle Into Same Set of Tubes

When setting collection parameters on the fraction collector, you can indicate that you want to collect each cycle into the same set of tubes.

For example, if you have a Code 0 (zero) rack installed, each sample will be collected into the same set of tubes per collection cycle. Collection will be made into the following tubes for each cycle.

Cycle	Tubes
1	1 - 80
2	1 - 80
3	1 - 80
4	1 - 80
etc.	etc.

#### Review or change rack

When the fraction collector "wakes up", it remembers and displays the last set of instructions it was given, for example:

Time mode 1.00 min/tube List Rack 0 Start

To display a description of the current rack, press the *Rack* soft key. You'll see:

Rack code \_\_ selected [description of rack] Ok

If a different rack is installed, enter the number code for the rack. The code for most racks is imprinted on the rack.

Press Ok.

#### Check tubes per cycle parameter

To indicate that you want to collect into the same set of tubes for each cycle, check that the *Tubes per cycle* parameter has been set to 0 (zero).

Press EDIT, then 2 to access the Technical menu. Now press the *Next* soft key until you see:

Tubes per cycle = \_\_\_ Next Quit

If the tubes per cycle is not set to 0 (zero), use the numeric keypad to change it.

#### Select time mode

Press the EDIT key. You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press the Time soft key.

#### Time per tube selection

You'll see the time selection display:

Time mode \_\_ min/tube Ok

Press the NO key to clear the display. Then enter the desired time per tube from 0.01 to 99.99 minutes. For this example, indicate 0.05 minute per tube.

Press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: \_\_to \_\_ Ok Add Del

Press Ok until the ready-to-run display appears. You're done setting parameters.

Time mode 0.05 min/tube List Rack 0 Start

## Example 2 Collection of Each Cycle Into a Different Set of Tubes

When setting collection parameters, you can tell the fraction collector to collect each cycle into a different set of tubes.

#### Review or change rack

When the fraction collector "wakes up", it remembers and displays the last set of instructions it was given, for example:

Time mode 1.00 min/tube List Rack 0 Start

To display a description of the current rack, press the *Rack* soft key.

You'll see:

Rack code \_\_ selected [description of rack] Ok

If a different rack is installed, enter the number code for the rack. The code for most racks is imprinted on the rack.

Press Ok.

#### Set tubes per cycle

To indicate that you want to collect into a different set of tubes for each cycle, access the Technical menu.

Press EDIT, then 2. Now press the *Next* soft key until you see:

Tubes per cycle = 0 Next Quit The *Tubes per cycle* parameter enables you to divide the rack into sections with a set number of tubes per cycle.

To collect each sample into a new set of tubes, use the numeric keypad to indicate a number greater than 0 (zero). Using the tubes per cycle and the total tube capacity for the rack, the number of cycles is calculated.

Note that if the number of tubes can't be divided into the tube capacity evenly, the number of cycles is the whole-number quotient; the remaining tubes are not used.

If you set 20 as the number of tubes per cycle for a code 0 (zero) rack, the number of cycles would be 4. Collection will be made into the following tubes for each cycle.

Cycle	Tubes
1	1-20
2	21-40
3	41-60
4	61-80

**Note**: The tubes per cycle option cannot be used in conjunction with the 203B's multiple collection head option. If you try to set the tubes per cycle option when the multiple collection head option is active, you will be prompted to change the *Multiple collect heads* parameter to single.

#### Select time mode

Press the EDIT key. You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press Time.

#### Time per tube selection

You'll see the time selection display:

Time mode \_\_ min/tube
Ok

Press the NO key to clear the display. Then enter the desired time per tube from 0.01 to 99.99 minutes. For this example, indicate 0.05 minute per tube.

Press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: \_\_ to \_\_ Ok Add Del

Press Ok until the ready-to-run display appears. You're done setting parameters.

Time mode 0.05 min/tube
List Rack 0 Cycle 1

## Coordinating with 712 HPLC Control Software

To control the components of the autopreparative HPLC system, use the Gilson 712 HPLC Control Software. Within the software, indicate the control and analysis conditions for the run. For detailed information on creating a method, consult your 712 HPLC Control Software User's Guide.

An example of a 712 method is listed on pages I-16 to I-17. You should also be aware of the following when setting parameters for the method.

#### Configuration

Do not include the injection pump in the Hardware Configuration box (Edit menu/Hardware Config. command). The pump information in that box is for mobile phase delivery pumps only because the software limits flow to the maximum flow rate of the smallest pump head size specified. Typically, the pumps in the system are arranged as shown below.

Pump	Unit ID	Function
A	1	Elution pump
В	2	Elution pump
C	4	Injection pump (not
		included in Hardware
		Configuration box)

#### **Number of Loops**

The number of Loop Passes set in the Run Conditions box (Edit menu/Run Conditions command) indicates the number of samples to be injected. This number is the same as the number of fraction collector cycles.

#### **Mobile Phase**

When setting up the method, indicate a schedule of timed flow events using the Mobile Phase box (Edit menu/Mobile Phase command). That schedule identifies the flow rate and percent composition that each elution pump contributes to the flow at various time points during the run. At the time of injection, set the flow rate from the elution pumps at 0.00 mL/min.

Following is a schedule for a gradient run. During injection (5.00 to 5.50 min.), Pumps A and B are not pumping mobile phase.

Time (min.,	Flow (mL/min.)	%B
0.00	30.000	5.0
4.99	30.000	5.0
5.00	0.000	
5.50	0.000	
5.57	30.000	5.0

At 4.99 min. into the run, the flow rate is 30.0 mL/min. with 5% coming from Pump B and 95% coming from Pump A. %A is calculated as 100% - %B.

Between 5.00 and 5.50 min., the elution pumps are not pumping. This is the time of injection.

At 5.57 min., Pumps A and B are once again pumping a total of 30.0 mL/min. with the percent composition remaining the same until the end of the run.

**Note**: You'll set up a GSIOC event that tells the injection pump when and how much sample to pump. You'll also set up a GSIOC event that checks the status of the injection pump and makes sure that 0 (zero) injection cycles remain. That is why there is a gap between time points 5.50 and 5.57.

See **GSIOC Events**, on the next page, for more information on setting up the required GSIOC events.

#### **Contact Events**

In the Contact Event Table (Edit menu/Contact Events command), set up an event that sends a pulse signal to the fraction collector. Send the signal after injection is completed. This starts the collection cycle set up on the fraction collector.

Time	Contact	Action	Description
6.00	1	Pulse	Sends a pulse
			signal via
			output 1 to the
			fraction
			collector.

Also, label Input A as "FC 203B event mark" if you have set up this connection as described on page I-2.

#### **GSIOC** Events

In the GSIOC Event Table (Edit menu/GSIOC Events command), set up events to:

- Lock out the front panel of the injection pump and enable the remote control function using the buffered L (Lock) command.
- Set the remote mode and lock the keypad for the fraction collector using the buffered **SR** (Set Remote) command.
- Dispense the sample using the injection pump. To do this, you'll use three buffered commands:
  - The **D** command sets the dispense mode.
  - The Eabcde command sets the dispense parameters.

To calculate the 3-digit number corresponding to abc, use this formula:  $abc = (dispense \ volume \ \div \ head \ size) \ x \ 1000$ . For example:  $abc = (5 \ mL \ \div \ 10) \ x \ 1000 = 500$ .

The parameter d is the refill speed of the piston and e is the dispense rate. To determine these, use the following table. Within the table, first locate the flow rate. Then find the corresponding refill speed and dispense rate. For example, if the flow rate is 5.0, the refill speed (d) is 4 and the dispense rate (e) is 3. See table below.

**Note**: The following table is for a 10 SC head. If you're using a 5 SC head, divide the flow rate you want to obtain by 2; then locate that flow rate in the table. If you're using a 25 SC head, multiply the flow rate you want to achieve by 2.5; then locate that flow rate in the table.

#### Dispense Rate for 10 SC head

	0	1	2	3	4	5	6	7	8	9
0	1.4	2.5	3.4	4.1	4.8	5.4	5.8	6.3	6.9	7.4
1	1.4	2.6	3.6	4.4	5.2	5.9	6.4	7.0	7.6	8.1
2	1.5	2.7	3.7	4.7	5.5	6.2	6.9	7.5	8.2	8.8
3	1.5	2.7	3.9	4.8	5.7	6.5	7.3	8.0	8.6	9.3
4	1.5	2.8	4.0	5.0	5.9	6.8	7.6	8.3	9.0	9.7
5	1.5	2.8	4.0	5.1	6.1	7.0	7.8	8.6	9.4	10.2
6	1.5	2.9	4.1	5.2	6.2	7.2	8.1	8.9	9.7	10.4
7	1.5	2.9	4.1	5.3	6.4	7.3	8.3	9.1	9.9	10.7
8	1.5	2.9	4.2	5.4	6.5	7.5	8.4	9.3	10.2	11.0
9	1.5	2.9	4.2	5.4	6.5	7.5	8.5	9.4	10.4	11.2

The buffered **B**n command indicates the number of times (n) to repeat the dispense cycle. If n is omitted, the default is 1 dispense cycle.

6.02

Read current tube number into 712 analysis report. For #, enter number from 1 to 9 to indicate report line on which tube number will be listed. (Note that line 0 is reserved for sample ID.)

- Request the status of the injection pump using the immediate P command. When the pump responds that 0 (zero) cycles remain, the computer continues the programmed method.
- (Optional) Read the current tube number into the 712 analysis report using the immediate T command. This will identify the tube at which the fraction collector started collecting the sample.

Refer to the following table to set up the GSIOC events for the above commands.

Time	Unit ID	Command Type	Command	Description
0.01	4	В	L	Lock out front panel of injection pump.
0.10	6	В	SR	Set the fraction collector in remote mode.
5.00	4	В	DE50043B1	Is the injection command for the injection pump:  D Sets the dispense mode.  E50043 Sets the dispense parameters (see description on previous page).  B1 Indicates the number of times to repeat the dispense cycle.
5.50	4	I	P	Request status of injection pump. When the pump responds that 0 (zero) cycles remain, the computer continues the programmed method.

T>#

#### **Method Listing**

Refer to the following method listing when setting parameters for an autopreparative

Listing of Method: \GILSON\712\_AUTO.USR\AUTOPREP.MTH

#### -Control Parameters-Run Time: 30.00 min

Loop Passes: 4

1.00 min Loop Begin 30.00 min Loop End Link Method : STOP

#### -Mobile Phase Events-

#### -Contact Events-

6.00 min Pulse 1 Start frac. coll.

#### -GSIOC Events-

0.01 min Buf\_Cmd to unit 4: L 'Lock injection pump front panel

0.10 min Buf\_Cmd to unit 6: SR 'Set frac. collector to remote mode

5.00 min Buf\_Cmd to unit 4: DE50043B1 'Set dispense speed and volume

5.50 min Imm\_Cmd to unit 4: P 'Read cycle#; if NB=0, stop injection

6.02 min Imm\_Cmd to unit 6: T>1 'Read tube number into report

#### -Analysis Parameters-

Integration Start: 5.51 min Integration Time: 24.49 min Peak Width: 0.20 min Peak Sensitivity: 2.0 %

Analysis Channel: A Save the data

Save the data

mV Full Scale: 10

% Offset: 10

Chart speed is 10 mm/min Do not print the strip chart

- -Analysis Report-Area percent report Save the report Report unnamed peaks
- -Scaled Plot-Do not print scaled plot Plot Analysis Channel (A)
- -Rubber Stamp-
- -Analysis Peak Table-

none

- -Analysis Eventsnone
- -Method Setup-Number of Pumps: 2 Contact Unit ID: 63 Data Unit ID: 63

ID Headsize Refill Comp. Pump A 1 50 250 46 Pump B 1 50 250 99

- -Chronological List of Events-
  - 0.00 min Flow = 30.000 % B = 5.0
  - 0.01 min Buf\_Cmd to unit 4: L 'Lock injection pump front panel
  - 0.10 min Buf\_Cmd to unit 6: SR 'Set frac. collector to remote mode
  - 1.00 min Loop Begin
  - 4.99 min Flow = 30.000 %B = 5.0
  - 5.00 min Buf\_Cmd to unit 4: DE50043B1 'Set dispense speed and volume Flow = 0.000
  - 5.50 min Imm\_Cmd to unit 4: P 'Read cycle#; if NB=0, stop injection Flow = 0.000
  - 5.51 min Integration Start
  - 5.57 min Flow = 30.000 %B = 5.0
  - 6.00 min Pulse 1 Start frac. coll.
  - 6.02 min Imm\_Cmd to unit 6: T>1 'Read tube number into report
  - 30.00 min Loop End



# **Autopreparative Collection Controlled by 305 Pump**



In a Gilson autopreparative HPLC system, one high-pressure pump repetitively injects sample. The autopreparative system can be an isocratic or a gradient system and will include a fraction collector, such as the 203B.

To coordinate system components, you'll use a Gilson 305 Pump. Using the 305's software, you'll set mobile phase composition, identify injection pump parameters, and coordinate fraction collection on the 203B.

This appendix describes how to set up system components for the autopreparative collection of fractions. You'll find information on making rear panel connections, setting parameters on the fraction collector, and setting parameters on the 305.

Appendix J

#### **Connecting System Components**

This section describes the electrical connections you need to make between the 203B, 305, and detector.

#### **GSIOC** Connections

To control the injection pump, use a GSIOC cable to connect the controlling 305, elution pump(s), and injection pump. If necessary, refer to the 305 User's Guide for more information on making GSIOC connections.

#### **Contact Connections**

Use 2-conductor cable to make contact connections.

To remotely start the fraction collector, send an output signal from the 305 Pump to the fraction collector. Connect the fraction collector Input A to the 305 Output 1 (10-11 pair). Be sure to match ground connections.

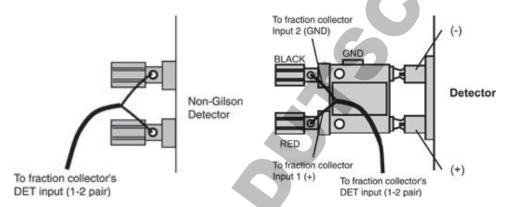
To mark tube advance directly on the data chart, connect the fraction collector event mark output (Output 1, 7-8 pair) to the appropriate input pair of the data unit (e.g., chart recorder, integrator, or non-Gilson software). Be sure to match ground connections.

If your data unit cannot accept input from an event mark output, you may connect the event mark output pair to the appropriate input pins of the detector. When set up this way, the data unit may interpret event marks as negative peaks on the detector trace.

#### **Detector Connections**

In addition, if collecting in peak mode, connect the detector to the fraction collector's DET input. (If you're using a Gilson detector, you'll need a Pomona cable adapter, part number 6374022611.) Connect the fraction collector's DET pin 1 to the detector's "+" output and the fraction collector's DET pin 2 to the detector's "-" (or GND) output.

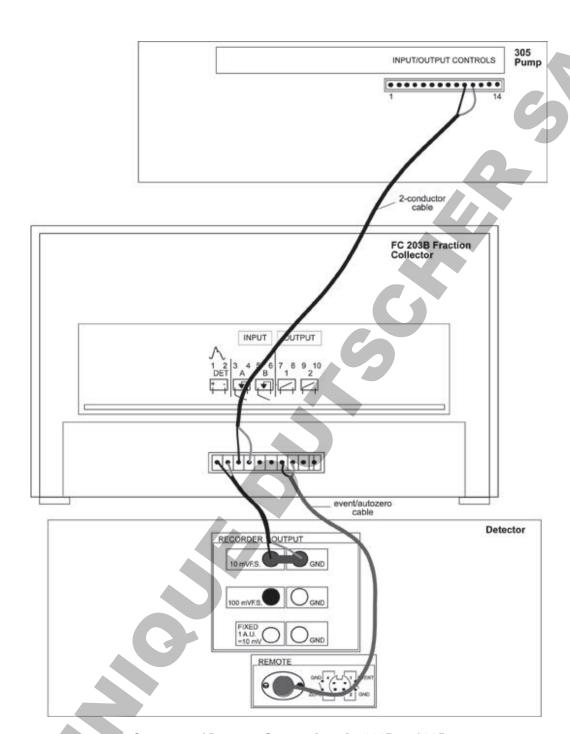
**Note**: If you're using a non-Gilson detector, you may need to connect the DET pin 2 to the metal screw in the lower left or right corner of the fraction collector to reduce ground noise. Refer to page 2-14.



Connection for a non-Gilson detector

Connection for a Gilson detector

Appendix



Contact and Detector Connections for 203B and 305

#### **Programming the 203B**

The 203B allows you to collect into the same set of tubes or into a different set of tubes in each cycle.

The following examples show how to set up parameters for a run using the time mode. One example collects each cycle into the same set of tubes while the other collects into a different set of tubes.

## **Example 1 Collection of Each Cycle Into Same Set of Tubes**

When setting collection parameters on the fraction collector, you can indicate that you want to collect each cycle into the same set of tubes.

For example, if you have a Code 0 (zero) rack installed, each sample will be collected into the same set of tubes per collection cycle. Collection will be made into the following tubes for each cycle.

Cycle	Tubes
1	1-80
2	1 - 80
3	1-80
4	1-80
etc.	etc.

#### Review or change rack

When the fraction collector "wakes up", it remembers and displays the last set of instructions it was given, for example:

Time mode 1.00 min/tube List Rack 0 Start

To display a description of the current rack, press the *Rack* soft key. You'll see:

Rack code \_\_ selected [description of rack] Ok

If a different rack is installed, enter the number code for the rack. The code for most racks is imprinted on the rack.

Press Ok.

#### Check tubes per cycle parameter

To indicate that you want to collect into the same set of tubes for each cycle, check that the *Tubes per cycle* parameter has been set to 0 (zero).

Press EDIT, then 2 to access the Technical menu. Now press the *Next* soft key until you see:

Tubes per cycle = \_\_\_ Next Quit

If the tubes per cycle is not set to 0 (zero), use the numeric keypad to change it.

#### Select time mode

Press the EDIT key. You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press the *Time* soft key.

#### Time per tube selection

You'll see the time selection display:

Time mode \_\_ min/tube

Press the NO key to clear the display. Then enter the desired time per tube from 0.01 to 99.99 minutes. For this example, indicate 0.05 minute per tube

Press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: \_\_to \_\_ Ok Add Del

Press Ok until the ready-to-run display appears. You're done setting parameters.

Time mode 0.05 min/tube List Rack 0 Start

# Example 2 Collection of Each Cycle Into a Different Set of Tubes

When setting collection parameters, you can tell the fraction collector to collect each cycle into a different set of tubes.

#### Review or change rack

When the fraction collector "wakes up", it remembers and displays the last set of instructions it was given, for example:

Time mode 1.00 min/tube
List Rack 0 Start

To display a description of the current rack, press the *Rack* soft key.

You'll see:

Rack code \_\_ selected [description of rack] Ok

If a different rack is installed, enter the number code for the rack. The code for most racks is imprinted on the rack.

Press Ok.

#### Set tubes per cycle

To indicate that you want to collect into a different set of tubes for each cycle, access the Technical menu.

Press EDIT, then 2. Now press the *Next* soft key until you see:

The *Tubes per cycle* parameter enables you to divide the rack into sections with a set number of tubes per cycle.

To collect each sample into a new set of tubes, use the numeric keypad to indicate a number greater than 0 (zero). Using the tubes per cycle and the total tube capacity for the rack, the number of cycles is calculated.

Note that if the number of tubes can't be divided into the tube capacity evenly, the number of cycles is the whole-number quotient; the remaining tubes are not used.

If you set 20 as the number of tubes per cycle for a Code 0 (zero) rack, the number of cycles would be 4. Collection will be made into the following tubes for each cycle.

Cycle	Tubes
1	1-20
2	21-40
3	41-60
4	61-80

**Note**: The tubes per cycle option cannot be used in conjunction with the fraction collector's multiple collection head option. If you try to set the tubes per cycle option when the multiple collection head option is active, you will be prompted to change the *Multiple collect heads* parameter to single.

#### Select time mode

Press the EDIT key. You'll see the mode selection display:

Select an edit path: Time Drop Peak Manl

Press Time.

#### Time per tube selection

You'll see the time selection display:

Time mode \_\_ min/tube Ok

Press the NO key to clear the display. Then enter the desired time per tube from 0.01 to 99.99 minutes. For this example, indicate 0.05 minute per tube.

Press Ok.

#### **Collection window option**

You'll see the collection window display:

Collect: \_\_ to \_\_ Ok Add Del

Press *Ok* until the ready-to-run display appears. You're done setting parameters.

Time mode 0.05 min/tube List Rack 0 Cycle 1

#### **Coordinating with 305 Pump**

You'll set up the 305 to control the mobile phase composition, control injection pump parameters, and coordinate fraction collection on the 203B. If necessary, refer to the 305 User's Guide for information on setting parameters and creating program files.

The pumps in the system are arranged as follows. Pump A is the controller and must be a 305. Pump B can be any model Gilson pump. The injection pump must be a 305, 306, or 307 if the controlling 305 is V1.2 or higher.

Pump	Unit ID	Function
A	1	Elution pump
В	2	Elution pump
Inj.	4	Injection pump

#### **Set Up Pump Hardware**

To set conditions for elution pump A, which is also the controller:

- Access the PUMP menu.
- Select pump A.
- Enter 125 ms for refill time.
- Enter compressibility of mobile phase pumped by pump A.
- Enter 50 SC for pump head size.

To set conditions for elution pump B:

- Access the PUMP menu.
- · Select pump B.
- Enter 125 ms for refill time.
- Enter compressibility of mobile phase pumped by pump B.
- Enter 50 SC for pump head size.

To set conditions for the injection pump:

- Access the PUMP menu.
- · Select pump Inj.
- Enter 125 ms for refill time.
- Enter compressibility of sample pumped by the injection pump.
- Enter 10 SC for pump head size.

#### **Set Up Contact**

In the I/O menu, set Output #1 to the open state.

#### Set Up Program File

In the File menu, create a program file for the run. Indicate the following in the file.

# of loops: number of samples to be injected

MIXT: <u>Time</u>	<u>% B</u>
0	5
30	5

FLOW:	Time	Flowrate
	0	30
	30	30

INJ:	<u>Time</u>	<u>Inj. Vol.</u>	Inj. Rate
	5	5 ml	5 ml/min

#### **Program Sheet**

Refer to the attached programming sheet when setting up parameters for the run.

#### 305 Programming Sheet

File Number: 3 Method Name: AutoPrep

#### SETUP PARAMETERS

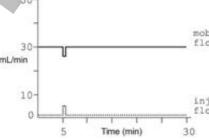
Pressu	re: High I	imit = 3000	Low lin	nit = 0	Units:	PSI
Numbe	r of pump	s= 3	Loops =	4 Li	nk file = 2	lone
Pump	Model	Solvent	ID No.	Refil	Comp.	Headsize
A	305	ACN	1	125	99	50
В	306	Water	2	125	46	50
С						
Inj.	306	Sample	4	125		10

#### **VO OPERATIONS**

Time	Contact	State	Function
5.1	OUT #1	Pulse	Start FC 204

# %B sample injection 5 Time (min) 30

COMPOSITION AND FLOW



#### PROGRAM STEPS

Step No.	Time	Event	Operation	Step No.	Time	Event	Operation
1	0	FLOW	30 ml/min	14			
2	0	MIXT	5% B	15			
3	5	INJ	Inject 5 ml	16			
4	5.1	TUO	Pulse OUT #1	17			
5	30	MIXT	5% B	18			
6	30	FLOW	30 ml/min	19			
7				20			
8				21			
9				22			
10				23			
11				24			
12				25			
13				26			



### **Collecting Sharp Narrow Peaks**



With the FC 203B Fraction Collector, peak detection is optimized for LC applications that collect peaks with a width at half height of 1 minute or longer. For HPLC applications that collect varying sizes of sharp narrow peaks, you may need to:

- connect the DET input of the fraction collector to the 10 mV full scale output of the detector, if the fraction collector is connected to the 100 mV full scale output.
- adjust the peak width and peak height values set in the fraction collector's software. Peaks with widths of approximately 0.4 minutes or less may require peak width and height settings that are two to five times greater than the average peak width and actual peak height. Consequently, the fraction collector may not be able to detect small peaks (5 to 10% of full scale) and large peaks (100% of full scale) within the same chromatogram.

The chromatograms on the next few pages show how to improve collection of sharp narrow peaks. The shaded peak areas and event marks identify what's being collected by the fraction collector.

In Figure 1, peak width and height are set as suggested in *Appendix H*, *Peak detection algorithm*. With those settings, only half of the large peak at 4.4 minutes is collected.

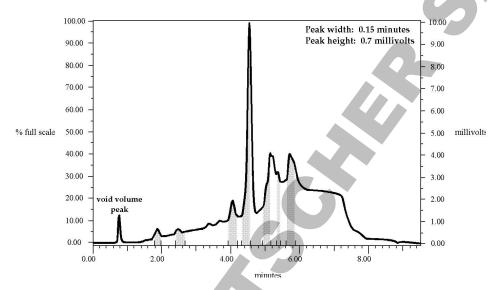


Figure 1

Next see how an increase in peak height affects peak collection. In Figure 2, the peak height is increased by more than five times the setting used in Figure 1, and peak width is not changed. The large peak is now collected, but most of the small peaks are missed.

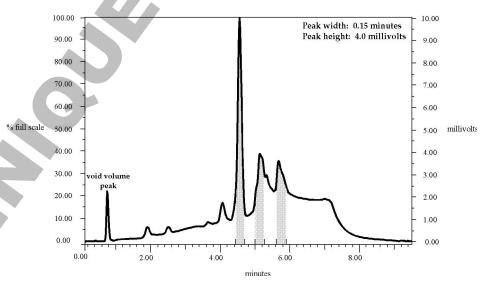


Figure 2

Finally see how an increase in peak width and peak height affect peak collection. In Figure 3, peak width is increased by more than four times the setting used in Figure 1. (Refer to the second bullet point on page K-1.) With these new values, more of the small peaks and all of the large peak are collected.

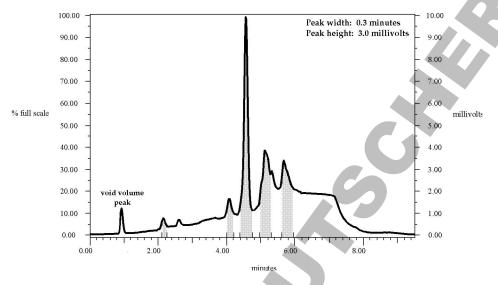


Figure 3

If your fraction collector is operating in a system controlled by Gilson UniPoint<sup>TM</sup> System Software, the peak width and sensitivity settings in the method can be used to determine the peaks detected by the fraction collector. These settings are specified in the Analysis Parameters and Analysis Events boxes. Consult the *UniPoint*<sup>TM</sup> System Software User's Guide for more information.



## **Replacement Parts and Accessories**

Part number Description 174732 Multiple column adapter 170730 3-way valve kit 709910206 2-conductor interconnect wire; 6 ft. Pomona cable adapter 6374022611 36078143 Shielded GSIOC cable, 30 in. 100731 Event cable to Gilson detectors 1707305 Small bore needle for Code 2 and Code 15 racks with 3-way valve Front drain trough 17013004 Inlet drop tubing assembly 1703548 1707303 Standard bore needle for 3-way valve

Inlet tubing assembly for 3-way valve, 2 ft.

Drain tubing assembly for 3-way valve, 2 ft.

Coupling for 1/4"-28 fittings; package of 5

Rack holder assembly for Code 0, 1, 2, 4, 11, and 14 racks

Tube retaining bar for Code 1 rack

Polypropylene pan

1307302

1307303

F1410050

1704731

170472

170471

es	17074304	Drain tubing for drain trough				
Replacement Parts and Accessories	638310512	10-pin terminal block connector				
	170350V	Drop detector assembly, Valox. Standard accessory.				
	170350	Drop detector assembly, Delrin. Delrin has strong chemical resistance to most organic solvents and most neutral-pH aqueous solvents. Not suitable for use with acids, bases, or oxidizing agents.				
	17235003	Replacement glass sleeves; package of 6				
	708038107	Power cord; 115V				
	7080316106	Power cord; 220V				
	6730054007	0.5 amp T-0.5 replacement fuse, Slo-Blo				