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Gilson
FC 204 Fraction Collector
User's Guide

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August 2002

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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.

Manufacturer's Address ..... 3000 W. Beltline Highway
Middleton, WI 53562

EC Office Address ..... Gilson S.A.S.
19 Avenue des Entrepreneurs, B.P. 145
F-95400 Villiers-le-Bel, France

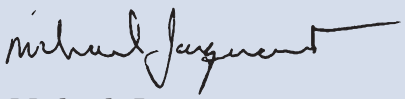
Type of Equipment ..... Laboratory Equipment

Model. ..... **FC 204 Fraction Collector**

Beginning with Serial Number: **179A6B001**

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

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Introduction 1

Welcome to improved fraction collecting. The Gilson FC 204 Fraction Collector is simple to use and straightforward to learn. Its sophisticated design makes it flexible enough to adapt to your collection goals. It is designed to make every operation clear and efficient.

There are four sources of information about the fraction collector:

- The **HELP** key on the fraction collector's keypad offers specific, on-line instructions during setup and operation.
- The *Gilson FC 204 Quick Reference Guide* answers key points quickly. Two copies are included.
- This user's guide describes how to operate the fraction collector.
- **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 204 Fraction Collector via UniPoint, you can obtain the following technical notes from your Gilson representative: *Fraction Collector Control* (part number LT21373) and *Autopreparative System Control* (part number LT21375).



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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 the 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:

- 3-Way Valve Kit
- Tray Platform
- Polypropylene Tray
- Inlet Tubing, 0.031" (0.8 mm) ID, with two Omnifit™-type end fittings
- Tygon Drain Tubing, 10', for drain trough and polypropylene tray
- Inlet Tubing Support Bracket
- Terminal Block Connector
- One spare fuse for 100/ 120V or two spare fuses for 220/ 240V
- Power Cord, 115V or 220V
- *Gilson FC 204 Fraction Collector User's Guide*
- *Gilson FC 204 Fraction Collector Quick Reference Guide* (2 copies)

Keep the original container and packing assembly in case the fraction collector must be returned to the factory.

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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 regional Gilson representative or call the Gilson Customer Service Department, toll-free 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.

To help us serve you quickly and efficiently, please refer to the **Before calling us** section on page 5-7.

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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.

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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/min. with large bore needle **or** Three-port, PTFE, 114 µL internal volume, 6 µL dead volume from the common port to the normally closed port, up to 200 mL/min. with large bore needle
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

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Manufacturing standards	Safety certification: • UL 3101-1 • CSA C22.2—No. 1010.1-92 • EN 61010-1 EMC certification: • EN 61326 • EN 61000-3-2 1995 • EN 61000-3-3 1995 • FCC Part 15
Maximum collection volume/tube	32 mL (18 x 180 mm tube) or 25 mL (18 x 150 mm tube)
Maximum fractions	Up to 768 fractions when configured with eight 96-well, 2 mL Whatman/Polyfiltronics microplates on a Code 17 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 18 columns with installation of optional multiple column adapter(s)
Number of racks	One Code 16 or 16D rack, one Code 17 rack, or up to four Code 20-Series racks (**all racks must be same code**)
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	47.9 x 46.4 x 33.0 cm (18.9 x 18.3 x 13.0 in.)

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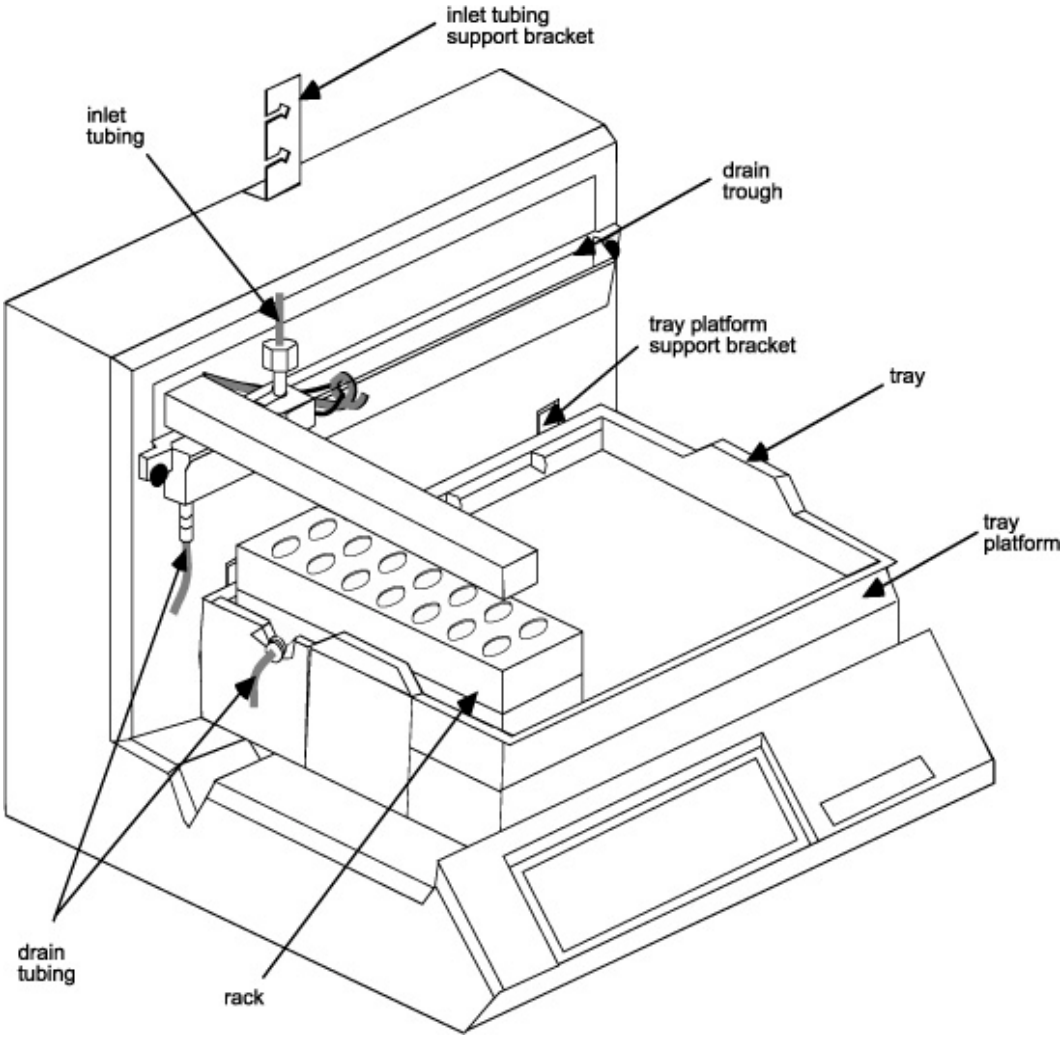
Power requirements	Frequency: 50 to 60 Hz Voltage: 100–120V or 220–240V, mains voltage fluctuations not to exceed ±10% of the nominal voltage Current rating: 0.5A for 100–120V or 0.25A for 220–240V
Programmable time units (min.)	From 0.01 to 99.99 minutes per tube, with 0.01 minute limit of resolution
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	100–720 ms, center-to-center, depending on rack type
Weight	10.9 kg (24 lbs.)
Zero-power memory	Maintains memory for a minimum of five years from date of shipment

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Installation 2

Plumbing Setup

Throughout the plumbing setup, refer to the plumbing setup diagram shown below.



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Hardware

Tray platform

All of the racks except rack Code 22 require the use of the tray platform. Align the notches on the rear of the tray platform with the support brackets on the fraction collector chassis. Then lower the tray platform into place. Make sure it fits securely.

When removing the tray platform, first lift it straight up and then bring it towards you.

Tray

For all racks except the Code 22 Rack, set the tray in place so it rests on the tray platform.

With the Code 22 Rack, remove the tray platform and place the tray on the floor of the fraction collector. Note that the drain port should be at the left rear of the tray.

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Tube racks

The fraction collector will accommodate up to four racks of the same code. Place the following racks in the tray so that they are perpendicular to the back of the fraction collector.

Rack Code	Tube Size (mm)	Tube Capacity (mL)	Tubes per Rack	Total Number of Tubes
20	10 x 100	4.5	108	432
21	13 x 100	9	60	240
22	18 x 150	25	44	176
	18 x 180	32		
23	17 x 55	6.8	44	176
	17 x 65	8		
23W	15 x 45	4	44	176
24	28 x 60	20	14	56
28	10 x 65	3	108	432
	10 x 75	3.5		
29	12 x 65	3.5	60	240
	12 x 75	5		
29 LE	11 x 40	1.5	60	240
29 SE	7 x 30	0.5	60	240

Note: Code 22 Rack doesn't require tray platform.
Code 23W Rack accepts Waters WISP vials.
Code 24 Rack accepts scintillation vials.
Code 29 LE and 29 SE Racks accept Eppendorf vials.

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Microplate racks

The fraction collector will accommodate up to four 96-well standard microplates using the Code 16 Rack (part number 170416). Choose the Code 16D Rack (part number 170416D) for using four deep-well microplates (96-well, 2 mL capacity).

The FC 204 Fraction Collector will accommodate up to eight Whatman/ Polyfiltronics microplates (2 mL, 5 mL, and 10 mL) using the Code 17 Rack (part number 170418) and the Code 17 Rack Heightener (part number 170417).

Refer to ***Appendix A, Racks*** for Code 16 and Code 17 Rack installation instructions.

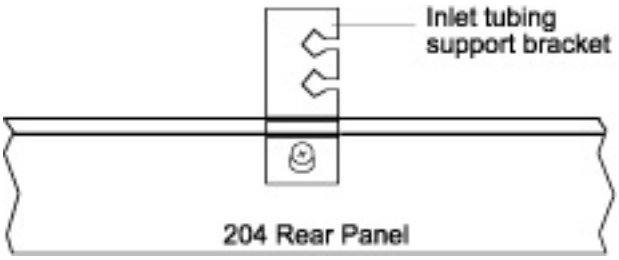
Both Code 16 and Code 17 Racks require a 3-way valve and small bore probe (part number 1707305) for collection into microplates.

Inlet tubing support bracket

Supplied with the fraction collector is the inlet tubing support bracket. After attaching the bracket to the back of the fraction collector, you'll use the bracket to support the inlet tubing connected to the dispense head.

Using a Phillips screwdriver, loosen the screw located at the top center of the fraction collector's rear panel.

Fit the keyhole in the bracket over the screw.



Seat the bracket over the screw and tighten the screw.

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3-Way Valve Installation

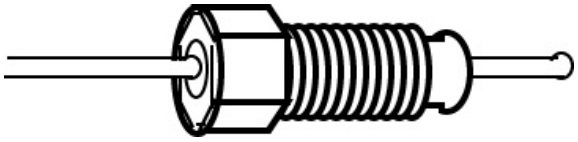
The 3-way valve supplied with the fraction collector is highly recommended for collection in either of the peak modes or when using collection windows. It prevents spillage when effluent is diverted to waste and simplifies collection of pure peaks.

Turn off power to the fraction collector using the MAINS power switch on the rear panel.

Gently pull the dispense head away from the drain trough.

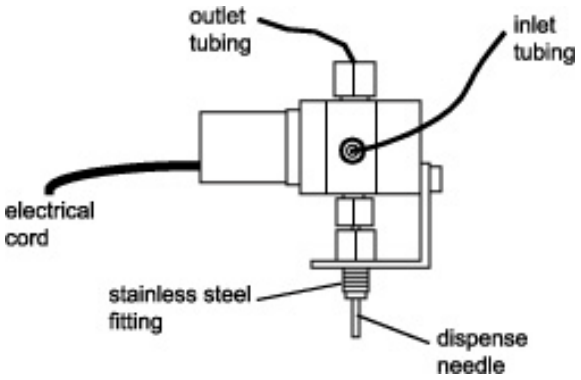
Inlet tubing

From the valve's accessories, locate the inlet tubing. It has Omnifit™-type fittings on both ends.



Omnifit™-type Fitting

Using the illustration below 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.



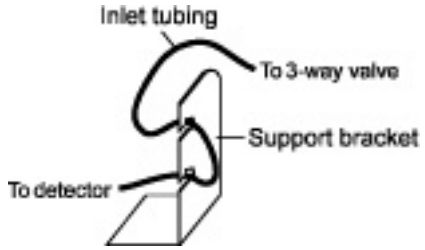
Finger-tighten the fitting. Pull gently on the tubing to make sure the fitting is firmly seated.

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Detector connection

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.

To support the inlet tubing, use the inlet tubing support bracket attached to the back of the fraction collector. The inlet tubing should form an "S" when inserted into the notches of the bracket.



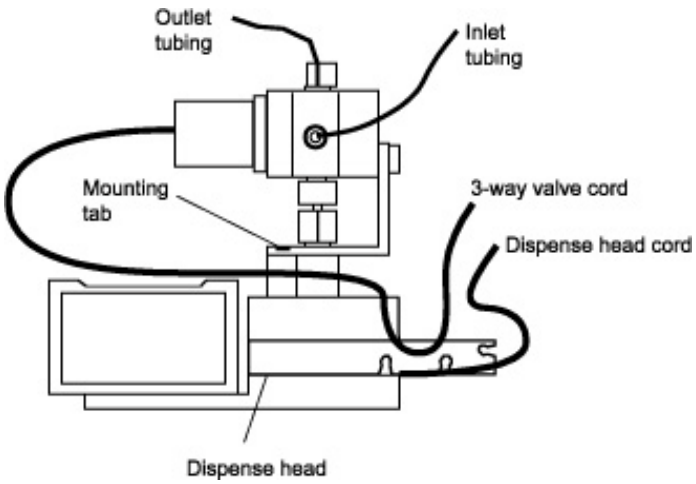
Outlet connection

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.

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Assembly

You are now ready to install the 3-way valve 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.

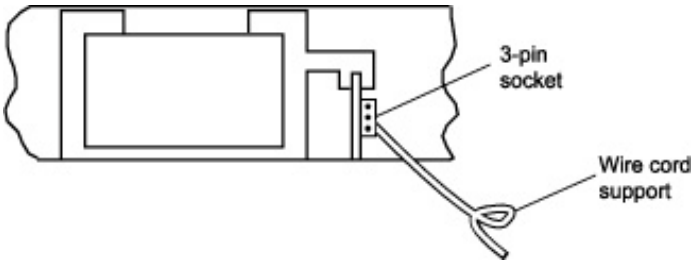
Install the 3-way valve by finger-tightening the stainless steel fitting into the dispense head. You may encounter some resistance after a few turns. If so, use a 5/ 16" open-end wrench to completely tighten the valve. Do not overtighten; tighten only until the assembly is stable.

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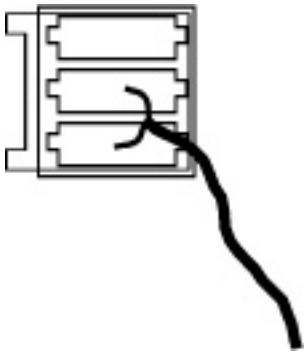
Electrical connection

Route the 3-way valve electrical cord through the notches on the dispense head bracket (see diagram on previous page). Then route the 3-way valve electrical cord through the wire cord support extending from inside the fraction collector. Use the plastic ties to encircle both the 3-way valve and dispense head electrical cords.

Now make the electrical connection for the 3-way valve. Locate the socket in the fraction collector into which you'll insert the plug from the 3-way valve. To do this, follow the wire cord support to the inside of the fraction collector. To the left of the support, you'll see a socket containing three pins.



Note that the plug from the 3-way valve has two small tabs on one side. Insert the plug into the socket with the two tabs facing towards the left.



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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. Liquid is diverted to drain through the 3-way valve outlet tubing. 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.

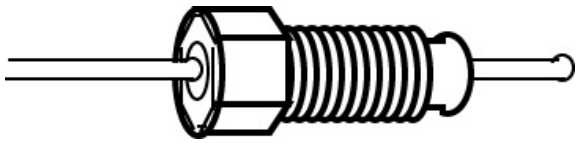
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Tubing

Inlet tubing

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

Using the following diagram, locate the inlet tubing. It has Omnifit™-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. Finger-tighten only.

Note: The length of tubing that extends from the fitting into the dispense head 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).

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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. But remember to leave enough tubing to account for movement of the dispense head.

To support the inlet tubing, you can use the inlet tubing support bracket attached to the back of the fraction collector. The inlet tubing should form an "S" when inserted into the holes of the bracket (see diagram on page 2-6).

The outlet tubing from Gilson detectors can be directly coupled to the fraction collector's inlet tubing, since both have Omnifit™-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).

Tray and drain trough connections

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. Connect one end of the other piece of tubing to the drain port of the tray and place the other end into a drain or container. These connections take care of collection spills or overfills that may occur during a run.

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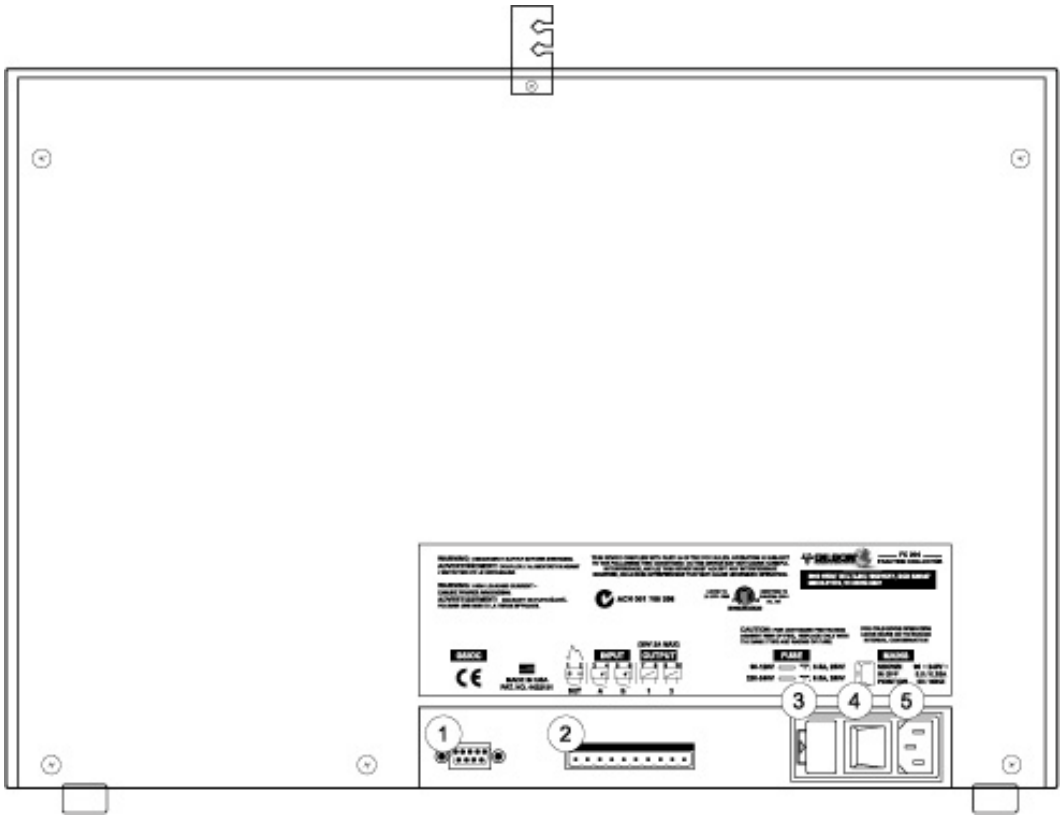
Electrical Setup

Rear Panel Description

Connections on the 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

Please read this entire section and refer to the rear panel diagram below before making any electrical connections. Do not turn on power to the fraction collector until all connections have been made.



Rear Panel Diagram

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Input/Output Port

You can take advantage of the powerful built-in features of your new fraction collector when you connect other equipment to the multi-purpose Input/ Output (I/ O) port.

The port has ten 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 (±10 mV to ±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 fraction collector can collect peak-containing fractions. Data from the detector can be digitized and sent to a controller for quantification of peaks.

- When you connect a remote switch contact to Input A (3-4 pair), other equipment can signal the fraction collector 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 fraction collector to end collection and home the dispense head.

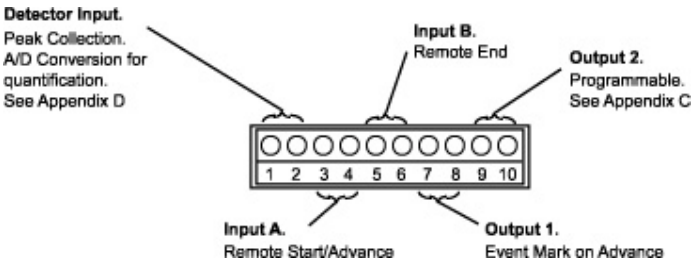
- When you connect your chart recorder's event mark input to Output 1 (7-8 pair), you can indicate tube advance on the chart.

- When you connect another device to programmable Output 2 (9-10 pair), the fraction collector can automatically start and stop that device.

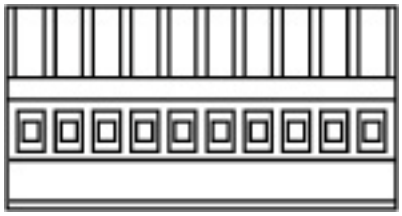
In the section called **Wiring to Other Equipment**, you will find information about how to make these I/ O connections work for you.

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The I/ O connection diagram 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 Input/ Output port you'll need:

- The terminal block connector (part number 638310512), included with your fraction collector accessories.
- A 2-conductor interconnect cable for each connection you'll make.

  Note: Use any 2-conductor cable with a gauge from 22–30 g. 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.
- A wire insulation stripper.
- A small-blade screwdriver.
- Additional items for connection to other devices; they'll be specified in the text.

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Making connections

To prepare a 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.

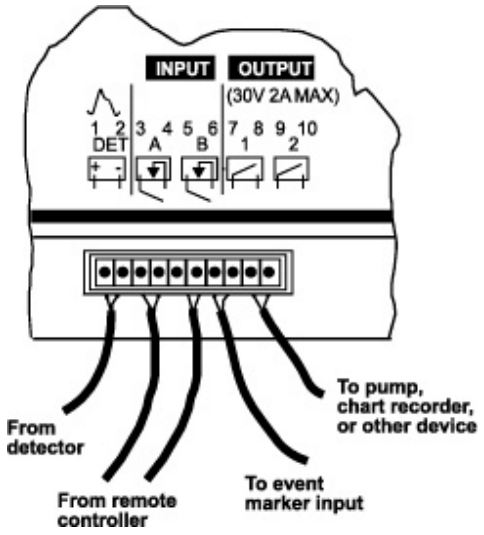
Note: Be sure to maintain the correct orientation of the terminal block connector relative to the multipurpose Input/ Output port. With the pin screws facing you and the wires entering from the bottom, the DET input is at the far left.

Note: Attach all wires before joining the terminal block connector to the Input/ Output receptacle on the fraction collector's rear panel.

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

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

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



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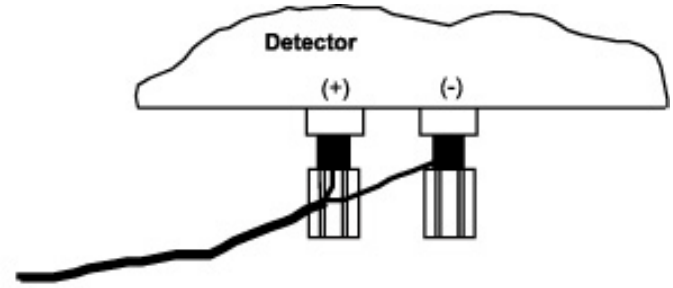
Wiring to Other Equipment

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.

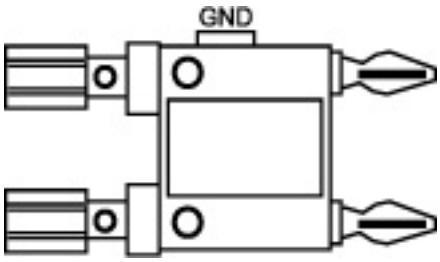
Detectors generally have one of two types of output connectors. Using the accompanying figures, determine which output connectors are used on your detector.

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 bare wire lead 1 to the "+" post on the detector and lead 2 to the GND (ground) post. Tighten the posts securely.



To 204 DET Input (1-2 pair)

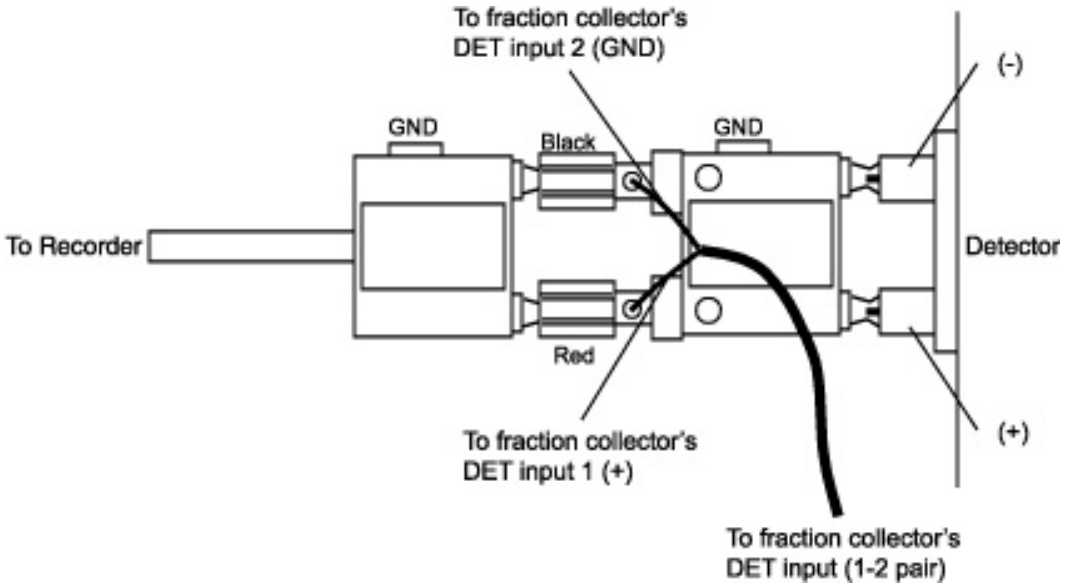
The other type of connector, like the one on most Gilson detectors, has fixed binding receptacles. To attach this type, you'll need a Pomona cable adapter (part number 6374022611) that looks like this.



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

Now you're ready to collect fractions using one of the peak detection modes. You're also set up to convert peak information into digital data for quantification by Gilson HPLC 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 ground or a correctly polarized TTL (0,5V) compatible signal (0 activates input).

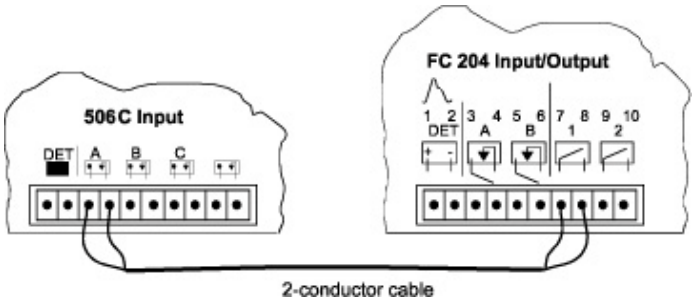
Full-function remote control can be achieved through the GSIOC port. See ***Appendix F*** for details.

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Event mark output at tube advance

You can mark tube advance directly on your data chart using the 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 Module and 712 HPLC System Control Software. In systems with a Gilson 506C System Interface Module, use a 2-conductor cable (part number 709910206) 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 or the Contact description box of a UniPoint Control Method, label Input A as the "FC 204 Event Mark", but don't enter any timed events for this input.

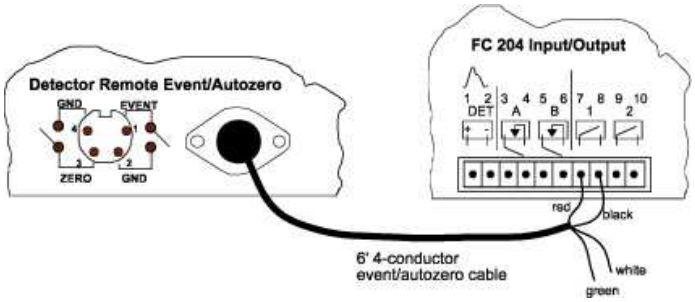


Event Mark Connection between FC 204 and 506C

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Gilson Detectors. In systems with a detector as the data unit, use the event/ autozero cable (part number 100731) 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 from being interpreted as a peak.



Event Mark Connection between FC 204 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 fraction collector 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 BD111/ 112 recorders available from Gilson, as well as most other Kipp & Zonen recorders, can accept input from the fraction collector event mark output.

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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.

1 Make the event mark at tube advance connection between the fraction collector and your data unit as described on page 2-19.

2 Short Output 2 (9-10 pair) to Output 1 (7-8 pair) of the fraction collector.

3 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

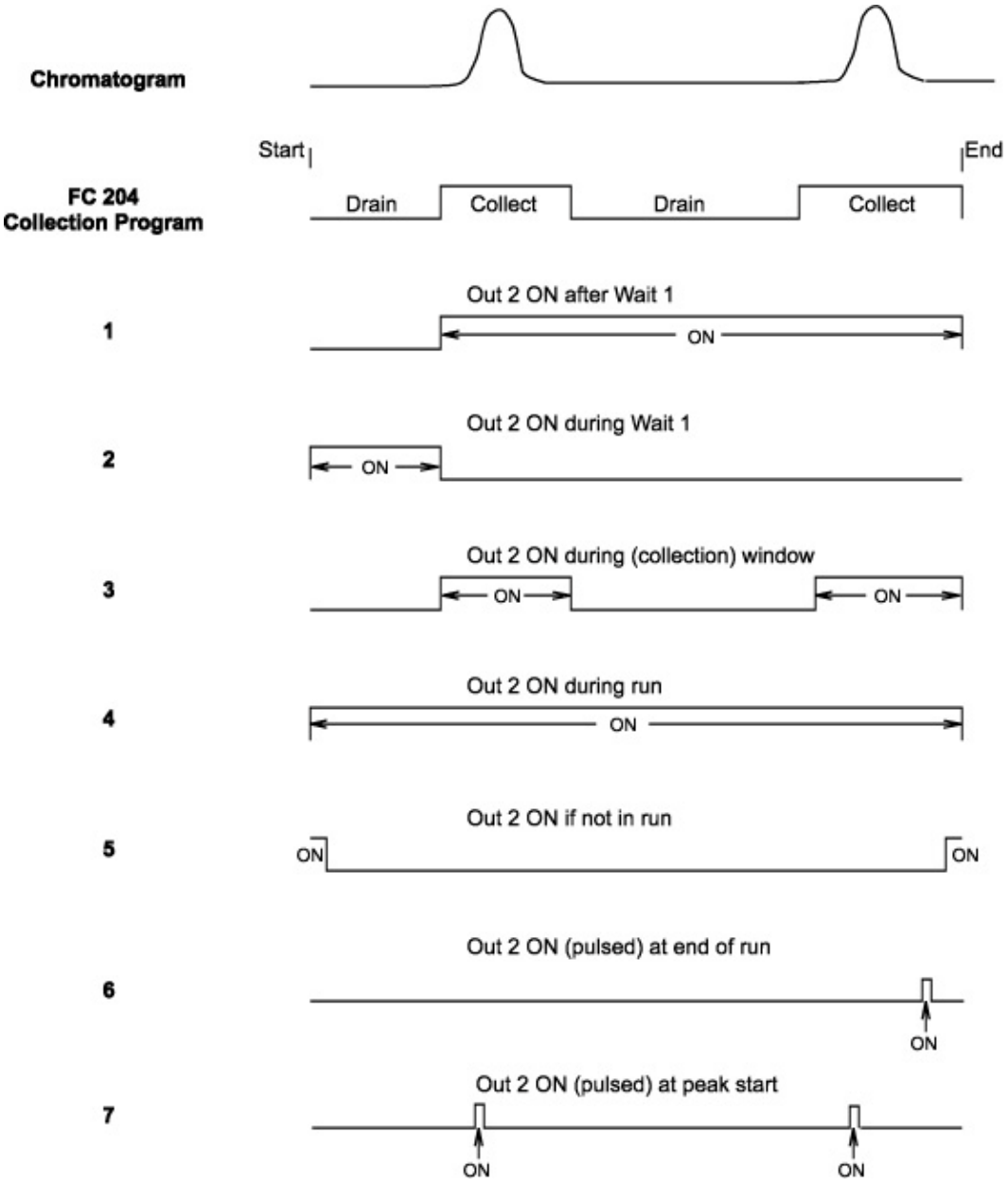
The fraction collector has several jobs to accomplish during normal operation. It may be waiting, draining, or collecting.

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 fraction collector is not in a run
6 ON (pulsed) at the end of a run
7 ON (pulsed) when a peak is detected.

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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.

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Then to specify which one of the seven options to use, access the technical menu. To do so, press EDIT. Then press 2. You'll see:

GSIOC unit ID =
Next *Quit*

Page through these screens by pressing *Next*, until you see:

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

To select that choice, press END or *Next*. If you want one of the other choices, repeatedly press the *No* key 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.

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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 computer.

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

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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.

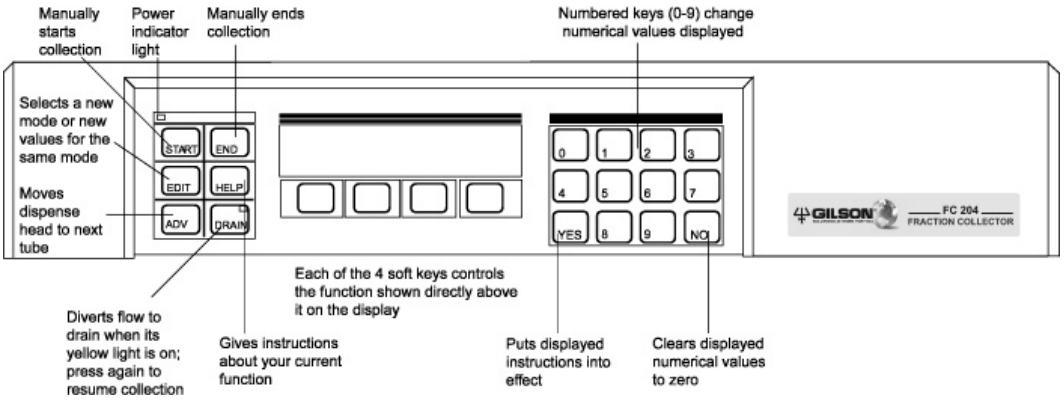
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Operation 3

The 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



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Front Panel

You give instructions to the fraction collector using the keys on the front panel.

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. Unlike soft keys, described below, their functions do not vary as you perform different operations.

You can start or end a run, advance the dispense head, drain the effluent, and edit the run options with these keys. You'll learn more about when and how to use each hard key in this manual.

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.

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Help Key

The most useful hard key may be the HELP key. It's always available, to give you specific help for the parameter you're currently setting.

To acquaint yourself with the HELP instructions, press the HELP key any time during setup. You can even press it during a run.

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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
Software version X.XX

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

__mode __min/tube
List Rack__ 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 load your tray and racks and press START. If the parameters need to be adjusted, read the next section, **Collection Options**.

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

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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.

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Review or Change Racks

The following table lists available racks and corresponding rack codes with a brief description of each.

FC 204 Software Rack Code	Gilson Rack Code	Vessel Type
16	16	Four standard or deep-well microplates with a row collection pattern
18	16	Four standard or deep-well microplates with a serpentine collection pattern
19	29 LE or 29 SE	Capped Eppendorf vials
20	20	10 x 100 mm tubes (4.5 mL)
21	21	13 x 100 mm tubes (9 mL)
22	22	18 x 150 mm (25 mL) tubes or 18 x 180 mm (32 mL) tubes
23	23 or 23W	17 x 55 mm (6.8 mL) tubes, 17 x 65 mm (8 mL) tubes, or 15 x 45 mm (4 mL) Waters vials
24	24	28 x 60 mm (20 mL) scintillation vials
26	16	Four standard or deep-well microplates using multiple column adapter
28	28	10 x 65 mm (3 mL) or 10 x 75 mm (3.5 mL) tubes
29	29, 29 SE, 29 LE	12 x 65 mm (3.5 mL) tubes, 12 x 75 mm (5 mL) tubes, 0.5 mL Eppendorf vials, or 1.5 mL Eppendorf vials
32	17	Eight 24-well (10 mL) Whatman/Polyfiltronics microplates
33	17	Eight 48-well (5 mL) Whatman/Polyfiltronics microplates
34	17	Eight 96-well (2 mL) Whatman/Polyfiltronics microplates

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 style of rack, enter the number code for the new racks. The code assigned to a rack is imprinted on the rack. All four racks installed on the fraction collector must have the same rack code.

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

Put the selected racks in place and press *Ok*.

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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 (zero) for the tubes per cycle.

If you want to collect each sample into a different set of tubes, indicate a number greater than 0 (zero) 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**.

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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 (zero) 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, 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.

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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 tube. During a run, the current tube 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 fraction collector resumes collection in the current tube. A yellow light on the DRAIN key indicates that the fraction collector is draining.

While the fraction collector 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 fraction collector 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 fraction collector 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.

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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 fraction collector to begin each cycle. See page 2-17 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*

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Collect Into Same Set Tubes

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

Example. If you install four Code 21 racks and set 0 (zero) as the number of tubes per cycle, 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	1–240
2	1–240
3	1–240
4	1–240
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 (zero) for the *Tubes per cycle* parameter. Using the tubes per cycle and the total tube capacity for the four racks, the fraction collector 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.

Also be aware that collection will continue between racks for a cycle. Therefore, the number of tubes per cycle doesn't have to be evenly divisible by the number of tubes in one rack.

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Example 1. If you install four Code 21 racks and set 20 as the number of tubes per cycle, the number of cycles will be 12. Collection will be made into the following tubes for each cycle.

Cycle	*Rack*	*Tubes*
1	1	1–20
2	1	21–40
3	1	41–60
4	2	61–80
5	2	81–100
6	2	101–120

Example 2. If you set 45 as the number of tubes per cycle when using four Code 21 racks, the number of cycles will be 5. Collection will be made into the following tubes for each cycle.

Cycle	*Rack(s)*	*Tubes*
1	1	1–45
2	1 and 2	46–90
3	2 and 3	91–135
4	3	136–180
5	4	181–225

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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 re-displayed.

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, 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 fraction collector prompts you to change the *Tubes per cycle* parameter to 0 (zero).

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

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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 fraction collector 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 fraction collector 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.

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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.

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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 collection 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 fraction collector would wake up to this display.

Note: If a number greater than 0 (zero) 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, 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.

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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.

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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 collection 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 fraction collector would wake up to this display.

Note: If you set a number greater than 0 (zero) 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, 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.

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Choosing Peak + Time 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 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*.

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Non-peak collection option

You'll see either of two displays:

Nonpeak: __ min/tube
Ok Discard
(To collect non-peak fractions)

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.

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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*.

Refer to ***Appendix H*** and ***Appendix K*** for more information on optimizing peak detection.

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*.

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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 two 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 fraction collector. 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.)

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

Internal Diameter		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.

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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 collection 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 fraction collector 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 (zero) 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, 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.

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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*.

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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.

Refer to ***Appendix H*** and ***Appendix K*** for more information on optimizing peak detection.

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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 (0.01–99.99) 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.

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If you have a relatively stable baseline, set the level value at about two 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 fraction collector. 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 in.	mm	Volume 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*.

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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 collection 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 fraction collector would wake up to this display.

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Note: The fraction collector 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 (zero) 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, 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.

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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 fraction collector. 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*.

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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 collection 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.

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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 semi-automatically, 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.

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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 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.

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.

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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.)

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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.

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Building No Windows

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.

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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 fraction collector to discard the effluent for 2 minutes before it starts to collect fractions.

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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 Add

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

Collect from: 0.0
Ok

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.

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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.

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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.

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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.

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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.

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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 racks, tray, tray platform, and drain trough for cleaning.

If you have questions concerning the chemical resistance of the fraction collector, contact your local Gilson representative or call Gilson directly at 800-445-7661 or 608-836-1551. Outside the U.S., contact your area Gilson representative.

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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.

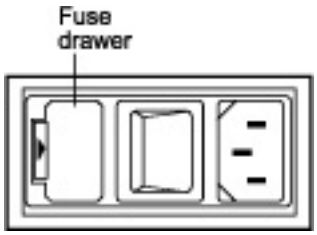
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Replacing a Fuse

To change a fuse, follow these steps.

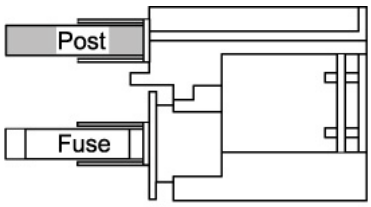
1 Disconnect the power cord from the power outlet and from the rear panel receptacle.

2 Locate the fuse drawer on the back panel.

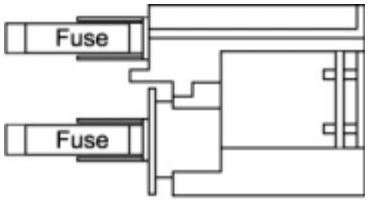


3 Insert a small screwdriver into the notch located to the left of the fuse drawer.

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.

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Troubleshooting 5

If you encounter a problem while operating the FC 204 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.

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Troubleshooting

Machine makes loud noise

- 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 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 the program matches the racks you are using.

- Check location of racks and tray; the racks may be out of position on the tray or the tray may not be seated properly in the tray platform.

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

- Check that the 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 it is still out of alignment, contact your local representative or the Gilson Customer Service Department.

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Drop detector not counting drops, or unreliable counting

- Check that the tubing in the dispense head's drop detector is not bent.
- Check that the tubing in the dispense head's drop detector is properly gauged.
- Be sure the inlet tubing fitting is completely tightened so the tubing cannot move up and down.
- Clean the glass collar in the drop detector (accessible from the underside of the dispense head; you will have to remove test tube racks from the unit). Use a cotton swab moistened with alcohol or water.
- Replace the glass collar in the drop detector if you are unable to clean the existing collar. Remove the two small screws from the underside of the dispense head. Lift the top piece off the bottom of the dispense head. Lift out the glass collar and install replacement. Be careful not to break the wires connected to the dispense head.
- Replace the dispense head assembly if faulty. Instructions on how to replace the dispense head are supplied with the replacement.
- Replace the electrical chassis.

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Separation problems in peak mode

- Check the peak parameters entered in the fraction collector program. Peak height should be the height of the smallest peak. Peak width should be the average width of the peaks in the chromatogram at half height.
- Specify negative peaks in the technical menu (EDIT 2 on keypad).
- Event mark on UV trace interfering with peak detection. Connect event mark to recorder on 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. The voltage should be less than 100 mV full scale.
- Check the peak parameters
- Check the polarity. Connections from detector output to fraction collector input may be incorrect.

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3-way valve not functioning or functioning incorrectly

- Check connection to the 3-pin socket on the circuit board, accessible through XY arm opening. Plugging or unplugging valve with the power on is not suggested as this may cause the machine to reset.
- Check that the connector is oriented properly.
- Check the fluid connections to the valve for proper orientation.
- Contact your Gilson representative or the Gilson Customer Service Department; the valve may be defective.

Input functions not operating

- Make sure connections into the terminal block connector are secure.
- Make sure the terminal block connector is secure in the Input/ Output port.
- Check the 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 the source supplying the input to the fraction collector is working.

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Output functions not operating

- Make sure connections into the terminal block connector are secure.
- Make sure the terminal block connector is secure in the Input/ Output port.
- Check the 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 from EDIT 2 loop.

Unit not operational

- Make sure the power is turned on.
- Check AC power cord connections
- Try a different AC outlet.
- Check fuse(s); replace if necessary.
- Replace the electrical chassis. Contact your local representative or the Gilson Customer Service Department.

Unit blows fuses

- Contact the Gilson Customer Service Department.

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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:

- the serial number and model number of the instruments involved. The serial number is visible on the back wall of the fraction collector when the tray platform is removed.
- the 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 fraction collector 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 Customer Service Department. 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.

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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

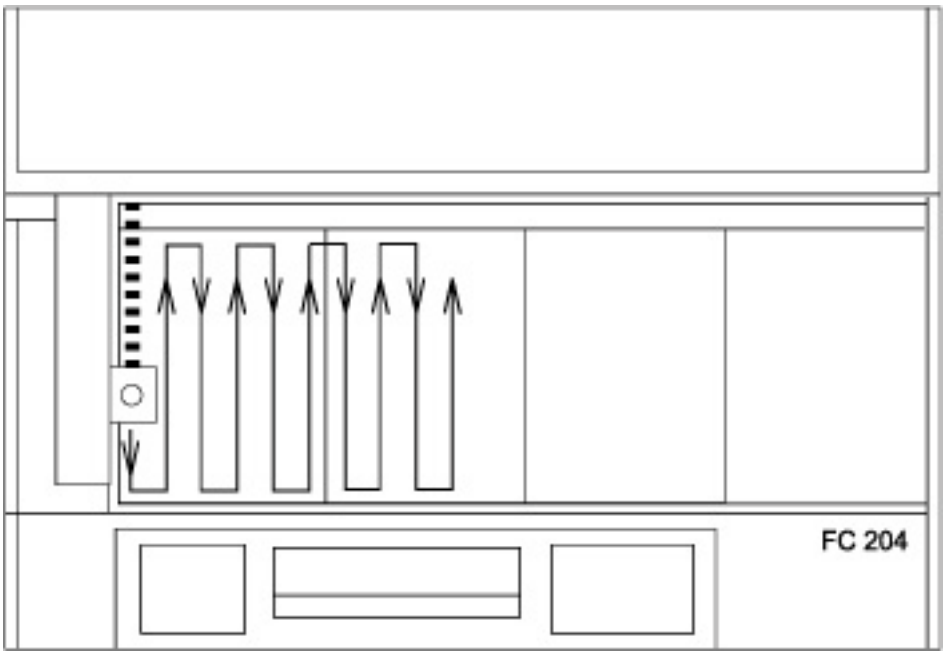
Outside the United States, contact your Gilson representative for return procedures.

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Racks A

The FC 204 Fraction Collector accommodates up to four racks of the same style. Each style of rack has its own rack code number. The fraction collector always needs to know the code number for the racks installed on it in order to accurately track the vessel positions for that rack.

Note: All of the racks except rack Code 22 require the use of the tray platform. For instructions on how to install the tray platform see ***Section 2, Installation***.



Collection Pattern
(test tube racks)

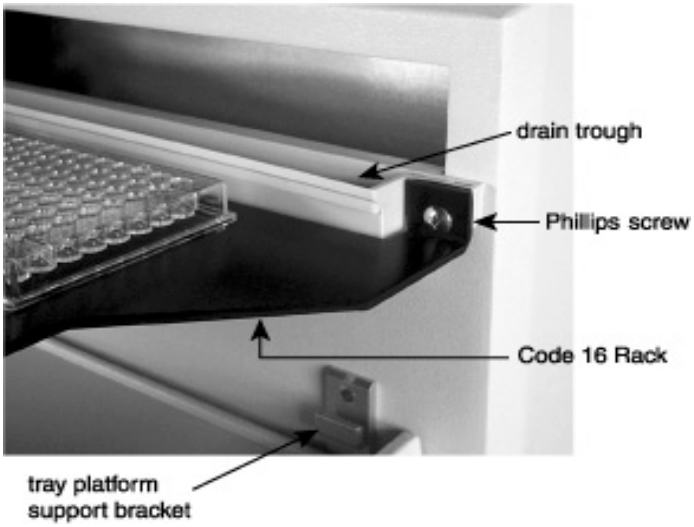
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Code 16 or 16D rack



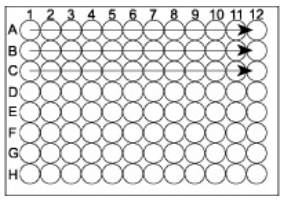
For four standard or deep-well microplates.
Material: aluminum
Vessel capacity: 0.3 mL per standard well
Part number: 170416 for standard well microplates
170416D for deep-well microplates

Installation: Remove the thumbscrews holding the drain trough in place. Align the rack and drain trough with the set of holes on the front standoffs of the fraction collector. Attach the rack and the drain trough using the Phillips screws supplied with the rack. Place the microplates on the rack horizontally.

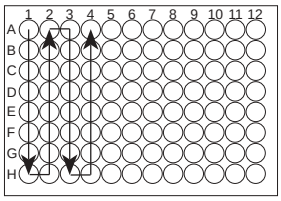


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Collection patterns: Refer to following diagrams and their related text.



Indicate rack code "16" in the software if you want to collect in a horizontal pattern



Indicate rack code "18" in the software if you want to collect in a serpentine pattern

To collect into standard or deep-well microplates using the multiple column adapter, indicate rack code "26" and set the multiple column adapter option in the EDIT 2 menu. See ***Appendix G*** for more information on this option

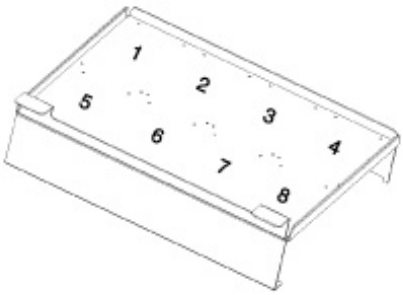
Note: A 3-way valve and small bore dispensing needle are required for collection into microplates.

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Code 17 rack and rack heightener



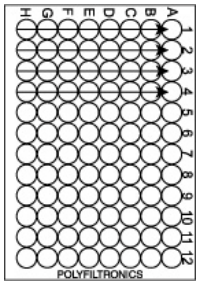
For eight Whatman/ Polyfiltronics deep-well microplates
Material: aluminum
Vessel capacity: 2 mL for 96-well microplates
5 mL for 48-well microplates
10 mL for 24-well microplates
Part number: 170418 for Code 17 rack
170417 for rack heightener



Installation: Install the tray platform as described in ***Section 2, Installation***. Fit the rack heightener on top of the tray platform using the tray platform support brackets (see the diagram on page 2-1). The lipped edge of the rack heightener should fit inside the tray platform. Install the microplates on the rack from left to right and top to bottom using the guide pins to hold them in place. The name on the microplate should be at the bottom of the microplate when it is in place on the rack. Slide the rack onto the heightener as far back as it will go. It will protrude slightly in the front. The drain trough must be removed to access the vessels accurately.

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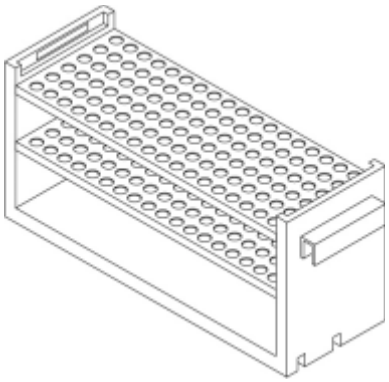
Collection patterns: Refer to diagram below for the collection pattern on each microplate and refer to the diagram on the previous page for the order of microplate collection.



Note: Indicate rack code “32” for 24-well (10 mL) microplates
Indicate rack code “33” for 48-well (5 mL) microplates
Indicate rack code “34” for 96-well (2 mL) microplates

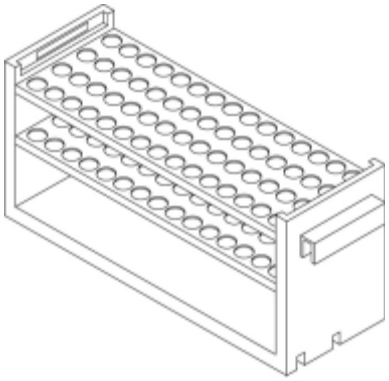
Note: A 3-way valve and small bore dispensing needle are required for collection into microplates.

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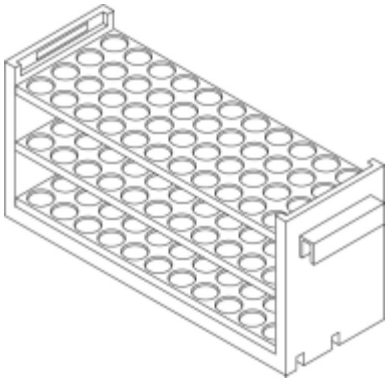
Code 20 rack

For 108 vessels.
Material: polypropylene
Vessels and capacity: 10 x 100 mm tubes (4.5 mL)
Part number: 150425



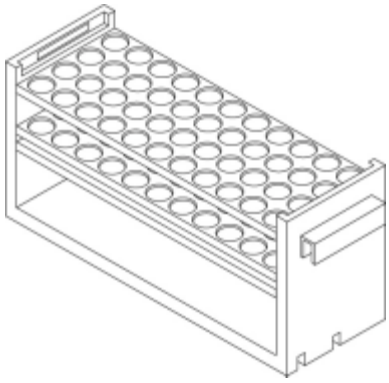
Code 21 rack

For 60 vessels.
Material: polypropylene
Vessels and capacity: 13 x 100 mm tubes (9 mL)
Part number: 150422



Code 22 rack

For 44 vessels.
Material: polypropylene
Vessels and capacity: 18 x 150 mm tubes (25 mL)
18 x 180 mm tubes (32 mL)
Part number: 150424



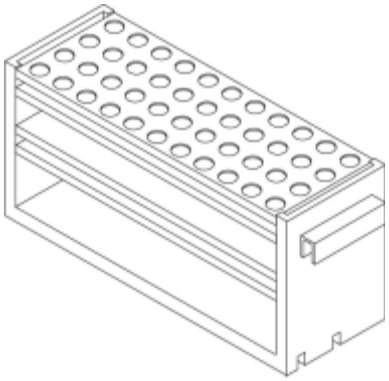
Code 23 rack

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

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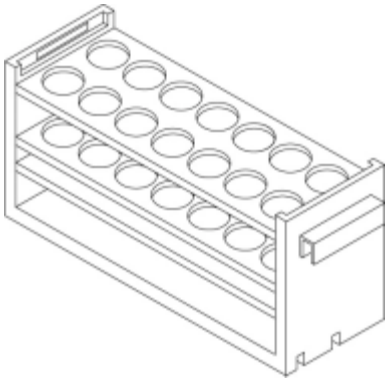
Code 23W rack

For 44 Waters WISP vials.
Material: polypropylene and stainless steel
Vessels and capacity: 15 x 45 mm (4 mL)
Part number: 270433



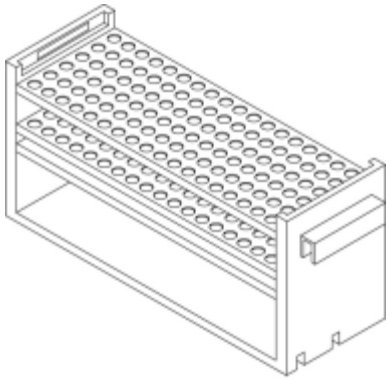
Code 24 rack

For 14 scintillation vials.
Material: polypropylene
Vessels and capacity: 28 x 60 mm (20 mL)
Part number: 150427



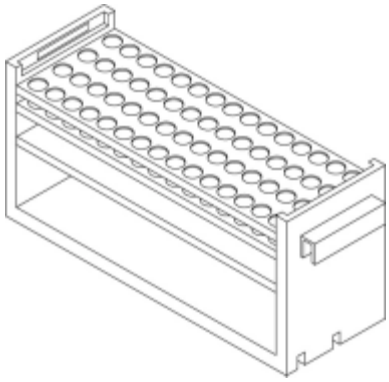
Code 28 rack

For 108 vessels.
Material: polypropylene
Vessels and capacity: 10 x 65 mm tubes (3 mL)
10 x 75 mm tubes (3.5 mL)
Part number: 150420

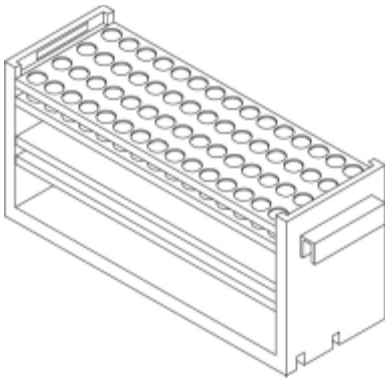


Code 29 rack

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

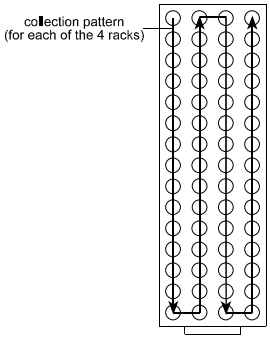


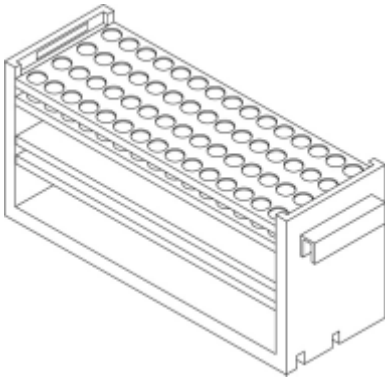
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Code 29 LE rack

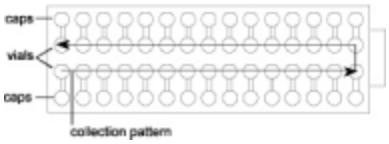
For 60 large (1.5 mL) Eppendorf vials.
Material: polypropylene and stainless steel
Vessels and capacity: 11 x 40 mm (1.5 mL)
Part number: 2704342
Note: When setting parameters in the software, indicate rack code “29”.





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”.

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Parameter Review Menu B

When you store parameters within any 204 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 FC 204 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.

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

Time mode __ min/tube
Ok

Drop mode __ drop/tube
Ok

Peak mode __ min/tube
Ok

Peak mode __ drop/tube
Ok

Nonpeak: discard (time)
Ok Collect
or
Nonpeak: __ min/tube
Ok Discard

Nonpeak: discard (drop)
Ok Collect
or
Nonpeak: __ drop/tube
Ok Discard

Peak width: _ min
Ok

Peak height: __ mV
Ok

Delay time: __ min
Ok

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.

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Technical Menu C

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

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GSIOC unit ID = 6
Next *Quit*

The unit ID identifies the FC 204 Fraction Collector to a master device (or a computer) when the two are connected via the Gilson Serial Input/ Output Channel (GSIOC). At the factory, Gilson set the unit ID 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 204s 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 fraction collector 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 fraction collector to the Gilson 506C System Interface Module, 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.

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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 and ***Appendix K*** describes how to collect sharp, narrow 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.

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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.

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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.

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The parameters in this menu are:

A/D slope: __
Next *Quit*

Detector A/D: *µV*
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 204v__.__
Next *Quit*

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Dispense Head End-Fitting Installation

E

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

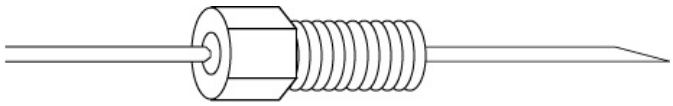
To ensure regular drop formation, you may have to reposition or replace the Omnifit™-type fitting or re-cut the end of the tubing.

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Replacement

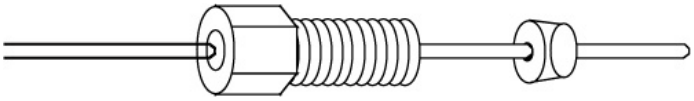
To replace the end fitting, use the following instructions:

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

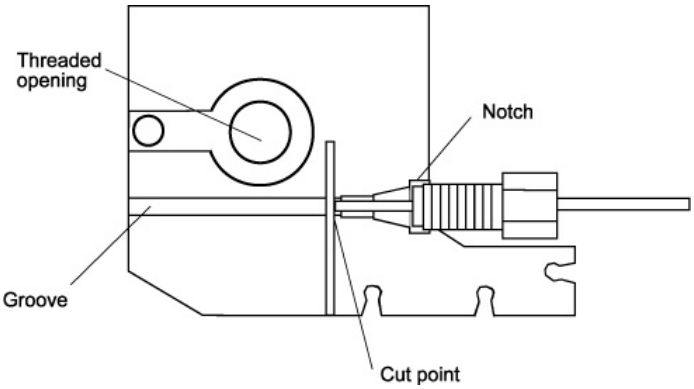


Replace the gripper so it rests about 3 cm from the tip. Use a needle nose pliers to pull the tubing through the gripper.

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.

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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.

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GSIOC Interface F

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

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 controller. The default ID code of the FC 204 Fraction Collector is 6. You can set it to any number from 0 to 63, but remember to use the same value on the controller when issuing commands to the FC 204 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 FC 204 Technical Manual.

To control the FC 204 Fraction Collector via the GSIOC interface, you will need the following equipment:

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

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GSIOC Commands

There are two kinds of commands that you can issue from your master unit to the FC 204 Fraction Collector.

- Immediate commands request status information from the FC 204 Fraction Collector. These one character, upper-case, or symbol commands are executed immediately, temporarily interrupting other GSIOC commands in progress.

- Buffered commands send instructions to the FC 204 Fraction Collector. These commands are executed one at a time.

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Immediate Commands

***** Reads the contact status of FC 204 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 204

? Reads contact status of FC 204 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 204 Fraction Collector

$ Resets the FC 204 Fraction Collector. It is equivalent to restarting the unit.

% This command reads the current version. Returns "204vx.x" where

x.x = currently installed software version

K Reads pressed keys. Returns "k" where:

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

R Reads both lines of the display. Returns "<line number 1> <line 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

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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 "xxx" where:

xxx = the current tube
000 indicates 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 moving
S if stationary

xxxx is in 0.1 mm units

O Reads FIFO 0. Format is defined by data transfer protocol.

9 Reads event FIFO. Returns "Xttttt" 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

ttttt = the time (in 10 ms units) since the last buffered 9 command or
000000 if the queue is empty

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Buffered Commands

C12 Closes contact output:

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

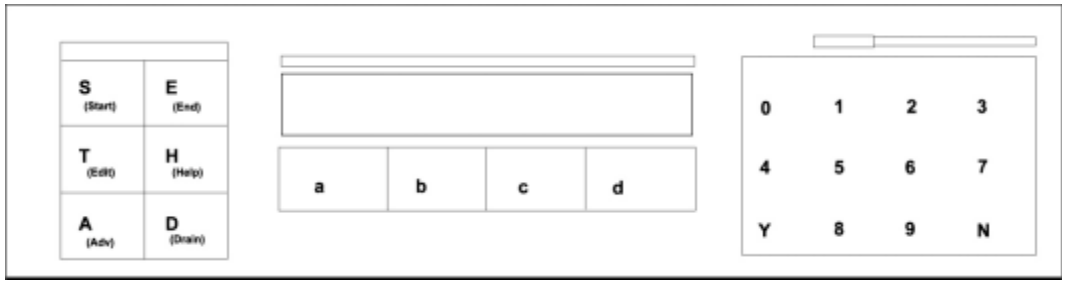
D12 Opens contact output:

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

Gddd Causes the FC 204 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 unlock front panel
1 for lock front panel

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Mxy Relaxes motors.

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

Warning: After you use this command, reset the FC 204 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 the parameter x, you can enter:

"p" to indicate a peak
"b" to indicate a baseline
"m" to show transition between merged peaks
"?" to have the FC 204 Fraction Collector resume peak collection

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

Vx Flips 3-way (diverter) valve:

x = 1 for divert to drain
0 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. Units are 0.1 mm towards the right.

Yxxxx Sets the Y-axis position. Units are 0.1 mm towards the front.

0xxxx Sets FIFO 0 sampling rate and clear 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.

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Multiple Column Option G

The 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 fraction collector rack configuration (except the Code 17 rack), in any operating mode. **To easily synchronize collection from several columns, you should 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(s)
- install the racks

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Setup

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 the column option display appears.

You'll see:

Single collection head
Next *Multi* *Quit*

or

Multiple collect heads
Next *Single* *Quit*

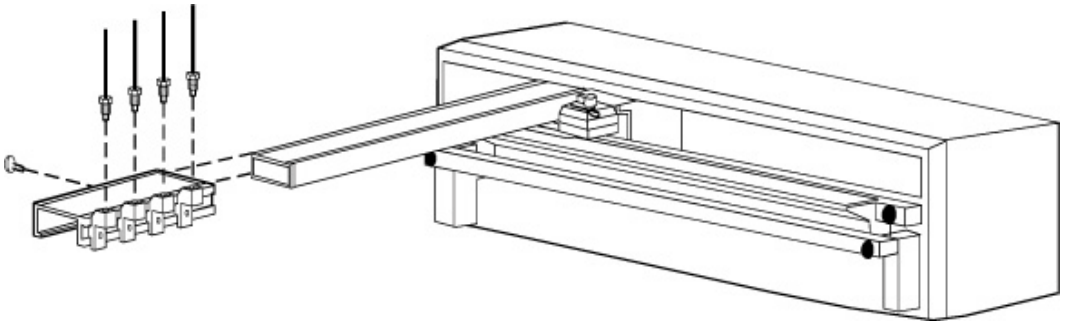
- If the first line shows *Single collection head*, press *Multi* to tell the fraction collector that you'll run multiple columns. Press *Quit* to return to the operating mode you selected.

- 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 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).

Note: To collect into standard or deep-well microplates using the multiple column adapter, indicate rack code 26 for the Code 16 or Code 16D Rack.

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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.

Note: If you want to collect from more than nine columns, you can position a second Gilson multiple column adapter (part number 174732) on the dispense arm. Slide the second adapter onto the arm until it touches the other adapter. Two multiple column adapters will fit on the dispense arm. Therefore, you can connect up to eighteen columns to the fraction collector

- If you will collect in manual, time, or peak + time mode, push the multiple column adapter(s) as far back as the adapter(s) will go. Tighten the thumbscrew(s).

- 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.

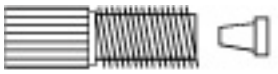
- If you choose to collect in a drop mode, position the drop detector over the tube closest to the rear of the fraction collector. Then push the multiple column adapter(s) back until the adapter touches the drop detector head. Tighten the thumbscrew(s).

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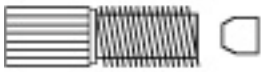
Connect Outlet Tubing

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

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 finger-tighten 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, Installation***.

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Install Racks

Select and install four racks with the same rack code, considering these two points:

- Fractions eluting from each separation column are collected in a single row from left to right across the width of all four racks. You must allocate one row for each chromatographic column.

- Each row must contain enough vessels to collect the desired number of fractions.

  Examples: Four Code 24 racks hold 56 scintillation vials arranged in a 7 x 8 array. With these racks installed, you could collect up to 8 fractions from as many as 7 columns.

  Four Code 28 racks hold 432 tubes arranged in an 18 x 24 array. With these racks installed, you could collect up to 24 fractions from as many as 18 columns.

- If a Code 16 or Code 16D rack is installed, indicate rack code "26" to collect into the microplates. Multiple column collection is not available for the Code 17 Rack.

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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.

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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 procedure 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. The dispense head will then move to the home position.
- remove the racks of tubes.

  Note: If you are using the programmable output 2 (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.

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Return to Single Column Collection

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

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.

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Peak Detection Using Slope or Millivolt Level

H

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.

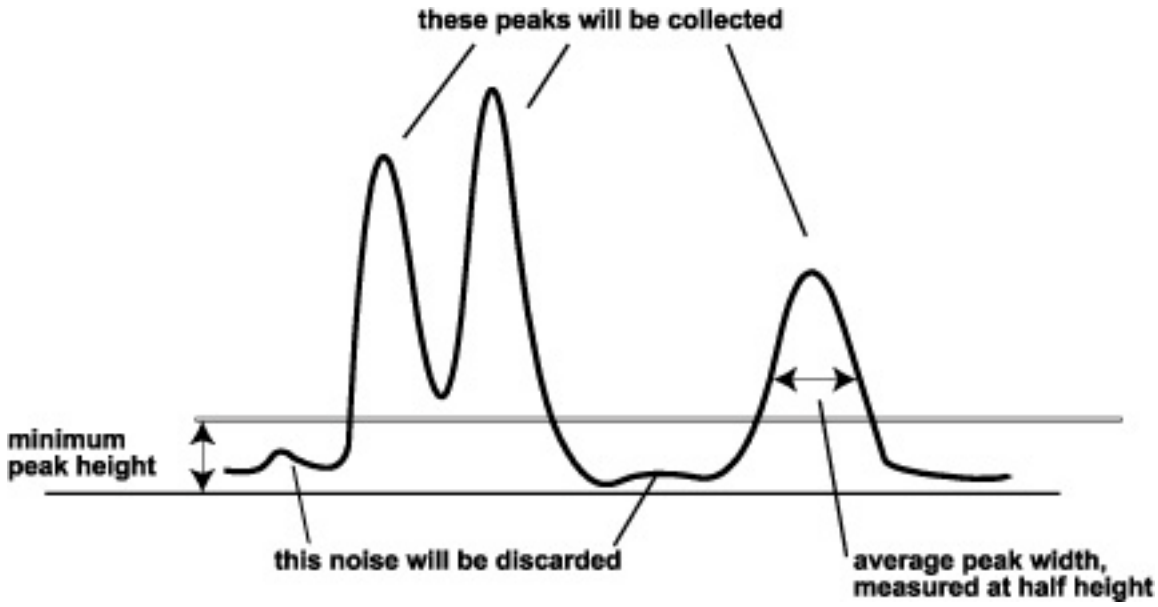
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Detection Using Slope (Peak Detection Algorithm)

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.



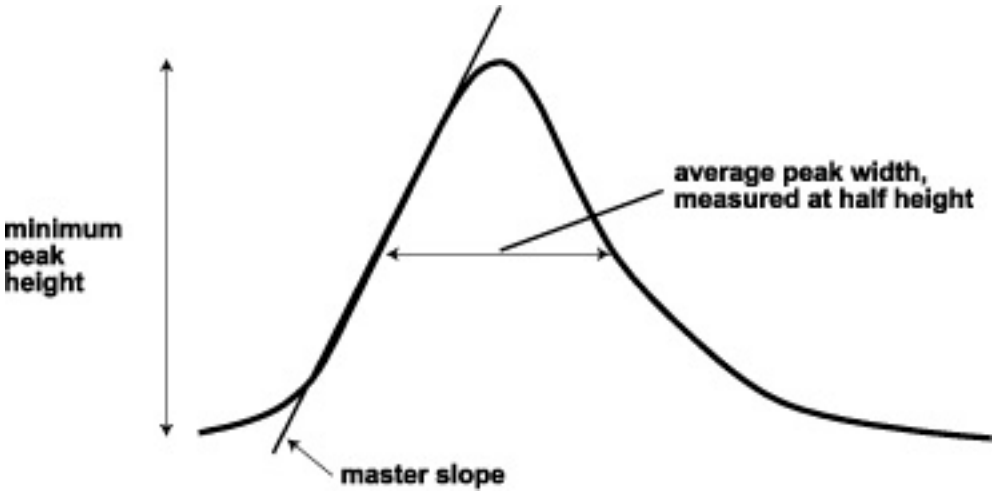
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Master Slope

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.

$$\text{Master slope } (S_0) = (k_0) \quad \frac{\text{Peak height}}{\text{Peak width}}$$

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



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Peak Phases

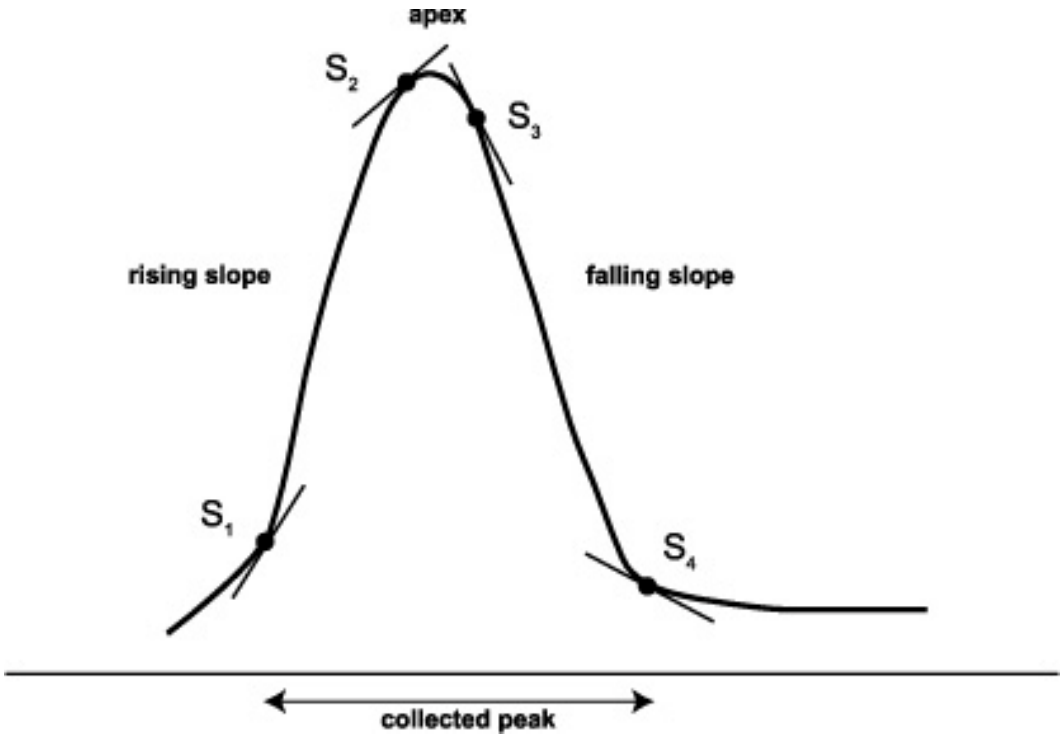
A positive peak is composed of three phases (refer to diagram below):

- 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_0:

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



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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_0 through k_5 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.

For instructions on how to collect sharp narrow peaks, refer to ***Appendix K***.

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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:

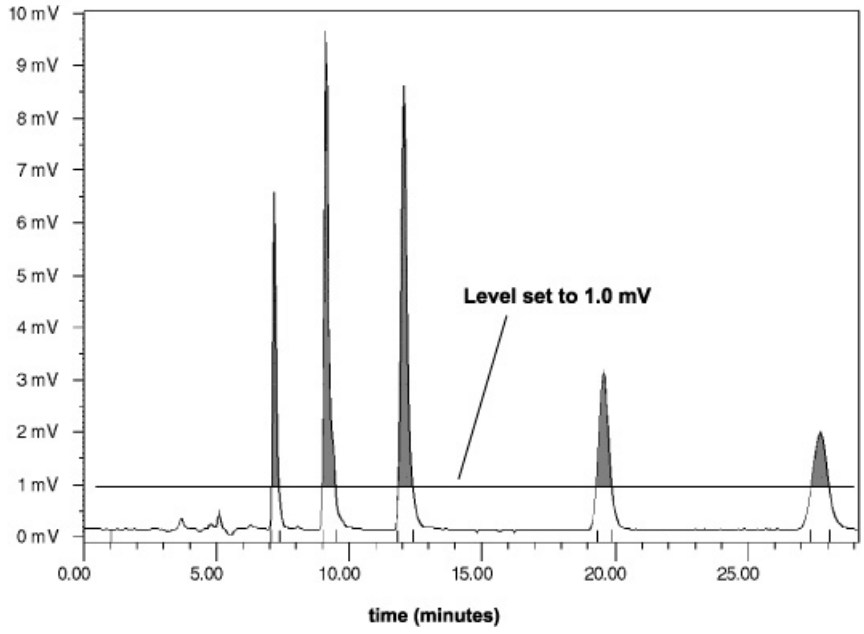
- width of an average peak, measured at half height
- mV value above which all peaks will be collected

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

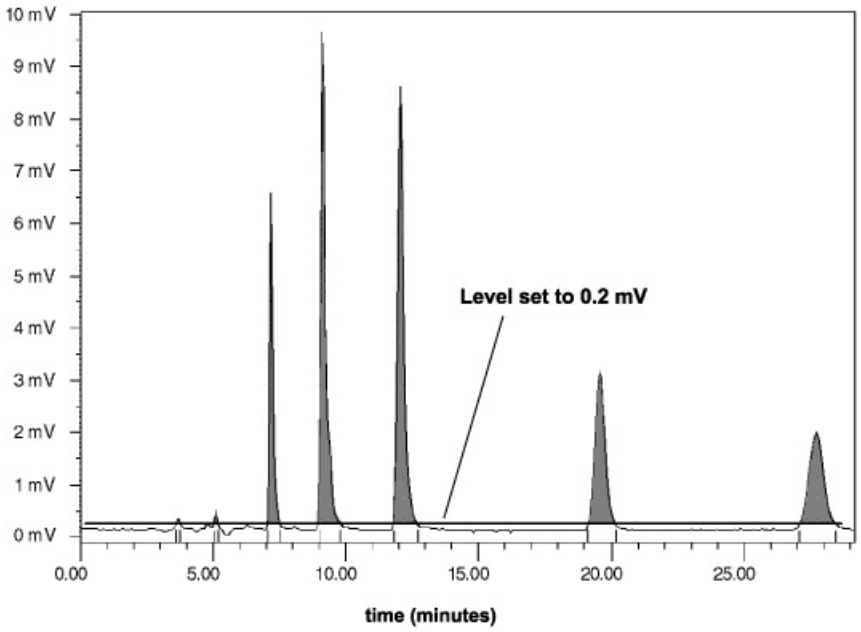
The fraction collector uses the mV value along with the peak width for peak collection. Peaks above the specified mV level are collected while peaks below the level are directed to waste. Peak width is used to filter noise, to determine the width of a safety window so the fraction collector software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

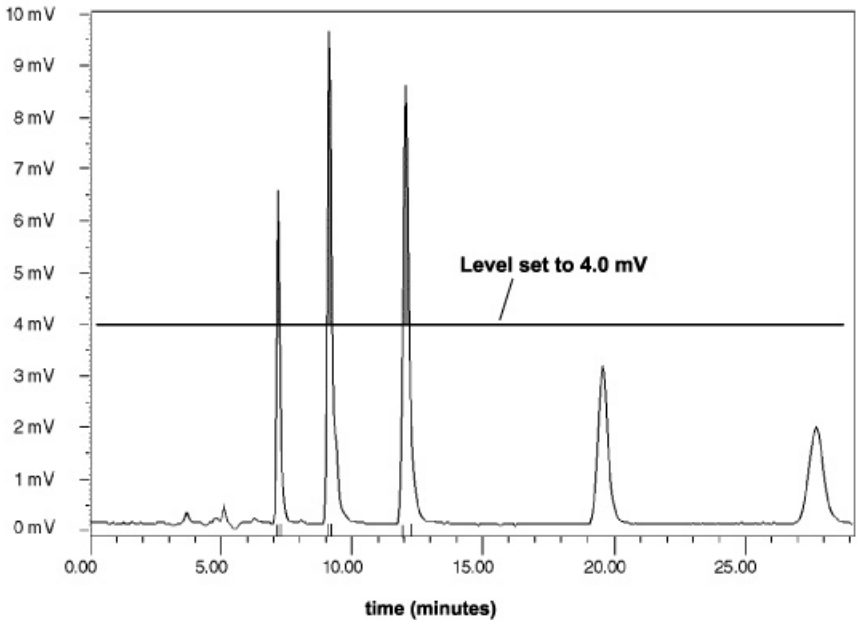
As a recommendation, set the mV level at two times the expected baseline fluctuation. For example, if the baseline stays between 0 and 1 mV during collection, set the level at 2 mV.

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.



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Autopreparative Collection Controlled by 712 HPLC Control Software

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 204.

To coordinate system components, you'll use Gilson 712 HPLC Control Software or UniPoint™ 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 *Autopreparative System Control UniPoint™ Technical Note* (part number LT21375) from your Gilson representative.

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Connecting System Components

This section describes the electrical connections you need to make between the fraction collector, the 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. Then connect the RS-232 cable to the serial ports of the 506C and the computer.

Contact Connections

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

To remotely start the fraction collector, you'll 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-14 describes how to set up the event to send the output signal.

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.

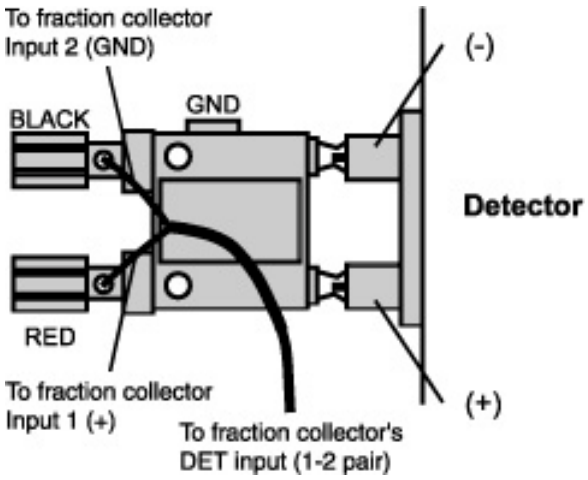
Note: If you're collecting in peak mode and also want an event mark at peak start, see page 2-20.

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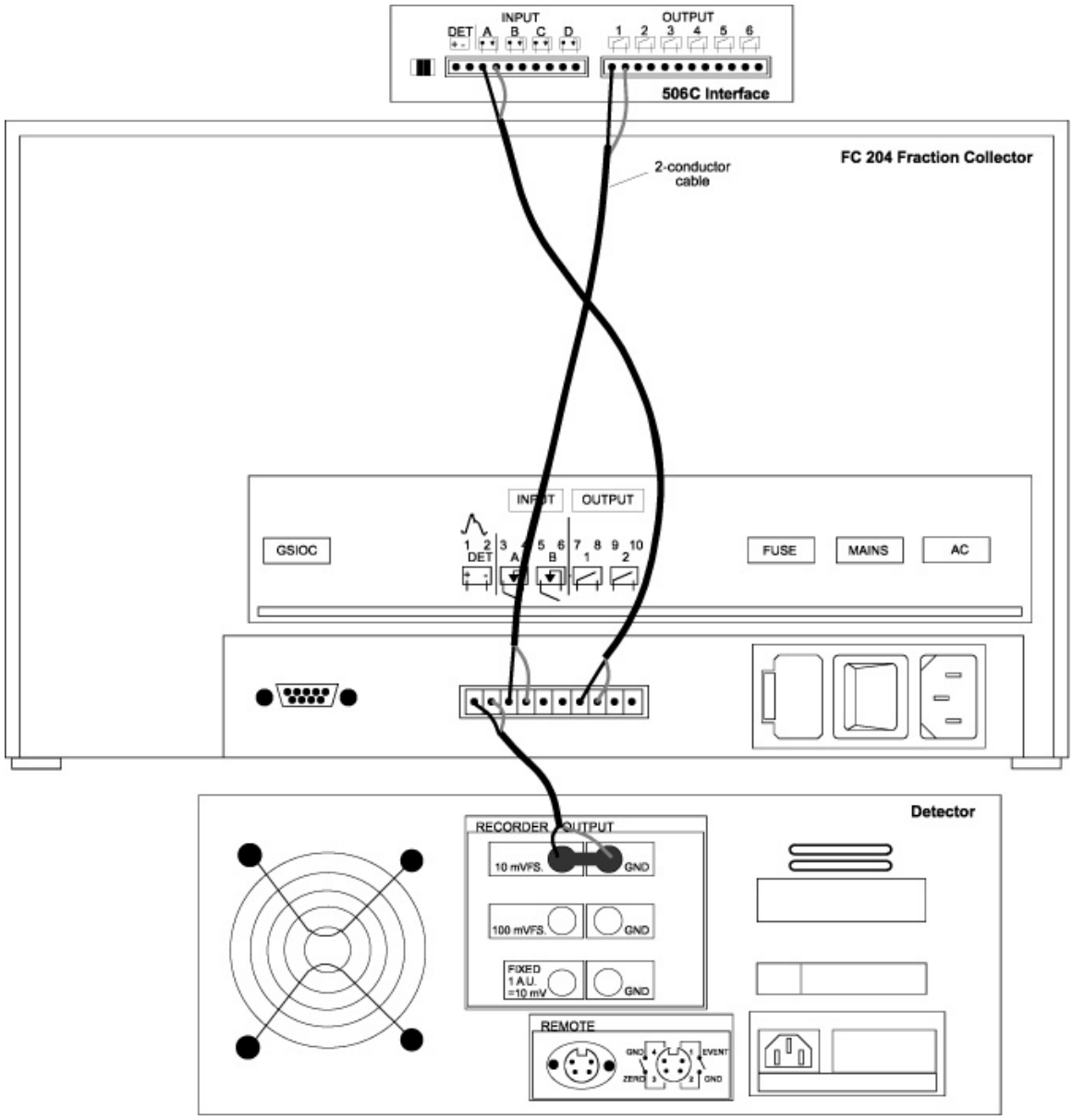
Detector Connections

In addition, if you plan to use the peak mode when collecting fractions, use the Pomona (or banana plug) cable in the detector's accessory package to connect the detector and fraction collector.

To make the connection, you'll also need a Pomona cable adapter (part number 6374022611). Make the connection as shown below using a 2-conductor cable. Be sure to 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 "GND" output.



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Contact and Detector Connections for 204 and 506C

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Programming the 204

The FC 204 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

By default, the fraction collector collects into the same set of tubes for each cycle. Therefore, each cycle begins at tube 1.

For example, if you install four Code 21 racks, collection will be made into the following tubes for each cycle.

Cycle	*Rack(s)*	*Tubes*
1	1	1–240
2	1	1–240
3	1	1–240
4	1	1–240
etc.	etc.	etc.

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Review/change racks

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

____ mode
List Rack __ Start

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

Rack code __ selected
[description of rack] Ok

If different racks are installed, enter the number code for the racks. The code is imprinted on the rack. Refer to ***Section 3, Operation*** for a complete listing of available racks and the corresponding code numbers.

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.

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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 min. per tube.

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)

Press *Ok*. You're done setting parameters.

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

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

Review/change racks

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

____ *mode*
List *Rack* __ *Start*

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

You'll see:

Rack code __ *selected*
[description of rack] *Ok*

If different racks are installed, enter the number code for the racks. The code is imprinted on the rack. Refer to ***Section 3, Operation*** for a complete listing of available racks and the corresponding code numbers.

Press *Ok*.

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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 racks 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. Using the tubes per cycle and the total tube capacity for the four racks, 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.

Also be aware that collection will continue between racks for a cycle. Therefore, the number of tubes per cycle doesn't have to be evenly divisible by the number of tubes on one rack.

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If you install four Code 21 racks and set 8 as the number of tubes per cycle, the maximum number of cycles will be 30. Collection will be made into the following tubes for each cycle.

Cycle	*Rack(s)*	*Tubes*
1	1	1–8
2	1	9–16
3	1	17–24
4	1	25–32
5	1	33–40
6	1	41–48
7	1	49–56
8	1 and 2	57–64
9	2	65–72
10	2	73–80
etc.	etc.	etc.

Note: The tubes per cycle option cannot be used in conjunction with the 204'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*.

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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/ tube from 0.01 to 99.99 minutes. For this example, indicate 0.05 min./ tube.

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)

Press *Ok*. You're done setting parameters.

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Coordinating with 712 HPLC Control Software

To control the components of the autopreparative HPLC system, use the 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-17 to I-18. 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
B	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.

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Mobile Phase

When setting up an autopreparative control 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.

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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 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), you 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/ head size) * 1000. For example: *abc* = (5 mL/ 10) * 1000 = 500.

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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

Refill Speed 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 Bn command indicates the number of times (n) to repeat the dispense cycle. If n is omitted, the default is 1 dispense cycle.

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- Request the status of the injection pump using the immediate P command. When the pump responds that 0 (zero) cycles remain, the system controller 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	B	L	Lock out front panel of injection pump.
0.10	6	B	SR	Set the fraction collector in remote mode.
5.00	4	B	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 system controller continues the programmed method.
6.02	6	I	T	Read current tube number into 712 analysis report.

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Method Listing

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

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—
0.00 min Flow = 30.000 %B = 5.0
4.99 min Flow = 30.000 %B = 5.0
5.00 min Flow = 0.000
5.50 min Flow = 0.000
5.57 min Flow = 30.000 %B = 5.0

—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 ‘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
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

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—Scaled Plot—
Do not print scaled plot
Plot Analysis Channel (A)

—Rubber Stamp—

—Analysis Peak Table—

none

—Analysis Events—
none

—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 'Read tube number into report
30.00 min Loop End

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Autopreparative Collection Controlled by 305 Pump J

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 204.

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 204.

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 204, and setting parameters on the 305.

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Connecting System Components

This section describes the electrical connections you need to make between the 204, 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, you'll send an output signal from the 305 to the fraction collector. Connect the 204 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 204 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.

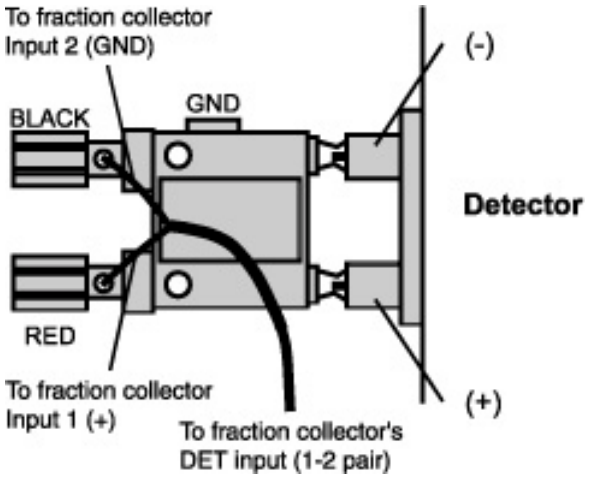
If your data unit cannot accept input from an event mark output, you may connect the event mark output pair to the appropriate input pins of the detector. When set up this way, the data unit may interpret event marks as negative peaks on the detector trace.

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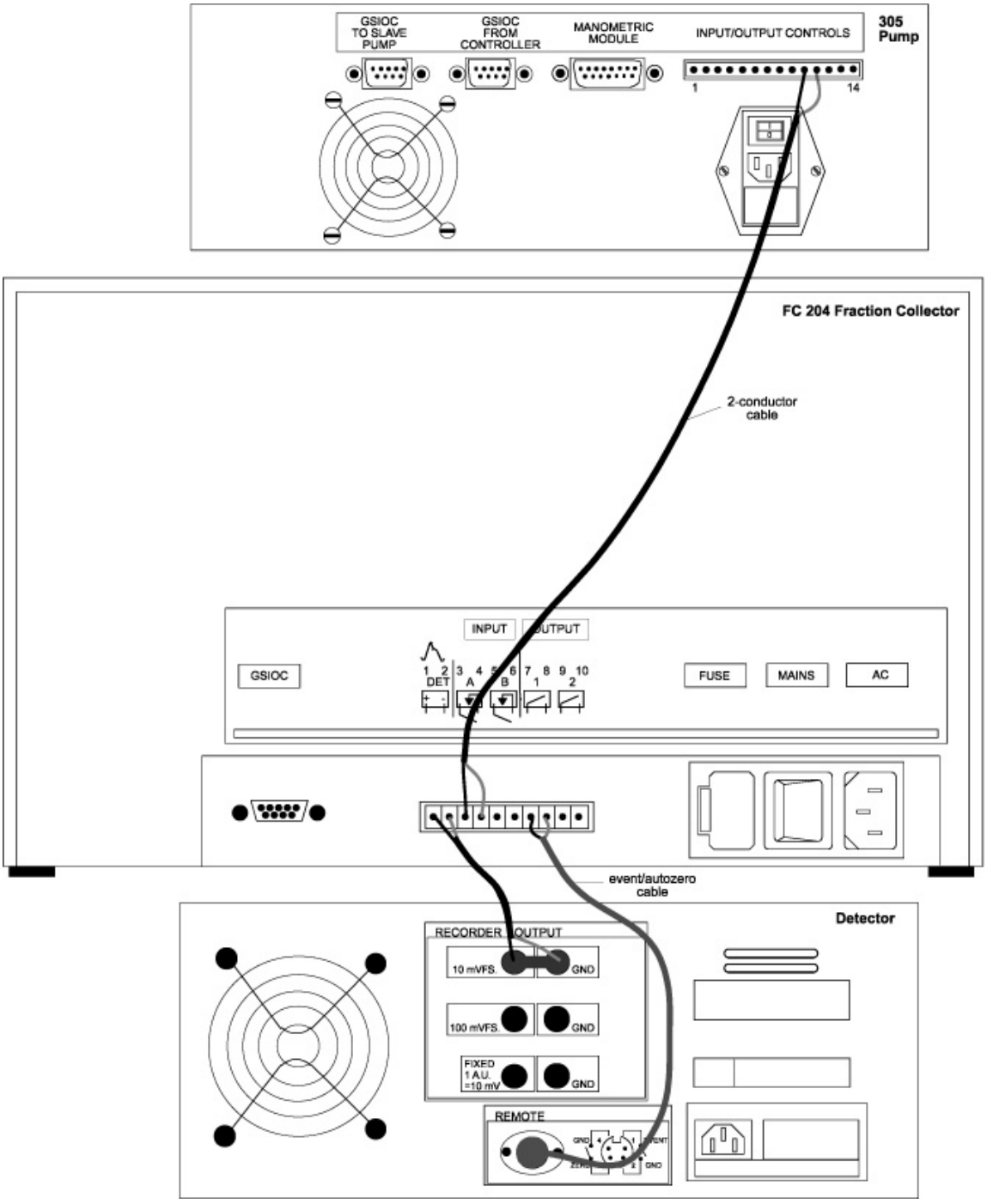
Detector Connections

If you plan to use the peak mode when collecting fractions, use the Pomona (or banana plug) cable in the detector's accessory package to connect the detector and fraction collector.

To make the connection, you'll need a Pomona cable adapter (part number 6374022611). Make the connection as shown below using a 2-conductor cable. Be sure to connect the 204's DET pin 1 to the detector's "+" output and the 204's DET pin 2 to the detector's "GND" output.



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Contact and Detector Connections for 204 and 305

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Programming the 204

The 204 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

By default, the 204 collects into the same set of tubes for each cycle. Therefore, each cycle begins at tube 1.

For example, if you install four Code 21 racks, collection will be made into the following tubes for each cycle.

Cycle	*Rack(s)*	*Tubes*
1	1	1–240
2	1	1–240
3	1	1–240
4	1	1–240
etc.	etc.	etc.

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Review/change racks

When the 204 "wakes up," it remembers and displays the last set of instructions it was given:

____ *mode*
List *Rack* __ *Start*

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

Rack code __ *selected*
[description of rack] *Ok*

If different racks are installed, enter the number code for the racks. The code is imprinted on the rack. Refer to ***Section 3, Operation*** for a complete listing of available racks and the corresponding code numbers.

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.

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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 min. per tube.

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)

Press *Ok*. You're done setting parameters.

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

When setting collection parameters on the 204, you can indicate that you want to collect each cycle into a different set of tubes.

Review/change racks

When the 204 "wakes up," it remembers and displays the last set of instructions it was given:

____ *mode*
List *Rack* __ *Start*

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

You'll see:

Rack code __ *selected*
[description of rack] *Ok*

If different racks are installed, enter the number code for the racks. The code is imprinted on the rack. Refer to ***Section 3, Operation*** for a complete listing of available racks and the corresponding code numbers.

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*

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The *Tubes per cycle* parameter enables you to divide the racks 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. Using the tubes per cycle and the total tube capacity for the four racks, 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.

Also be aware that collection will continue between racks for a cycle. Therefore, the number of tubes per cycle doesn't have to be evenly divisible by the number of tubes on one rack.

If you install four Code 21 racks and set 8 as the number of tubes per cycle, the maximum number of cycles will be 30. Collection will be made into the following tubes for each cycle.

Cycle	*Rack(s)*	*Tubes*
1	1	1–8
2	1	9–16
3	1	17–24
4	1	25–32
5	1	33–40
6	1	41–48
7	1	49–56
8	1 and 2	57–64
9	2	65–72
10	2	73–80
etc.	etc.	etc.

Note: The tubes per cycle option cannot be used in conjunction with the 204'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.

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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/ tube from 0.01 to 99.99 minutes. For this example, indicate 0.05 min./ tube.

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)

Press *Ok*. You're done setting parameters.

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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 204. 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
B	2	Elution pump
Inj.	4	Injection pump

Set Up Pump Hardware

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

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

To set conditions for elution pump B:

1 Access the PUMP menu.
2 Select pump B.
3 Enter 125 ms for refill time.
4 Enter compressibility of mobile phase pumped by pump B.
5 Enter 50 SC for pump head size.

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To set conditions for the injection pump:

1 Access the PUMP menu.
2 Select pump Inj.
3 Enter 125 ms for refill time.
4 Enter compressibility of sample pumped by the injection pump.
5 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:	Time	%B	
	0	5	
	30	5	
FLOW:	Time	Flowrate	
	0	30	
	30	30	
INJ:	Time	Inj. Vol.	Inj. Rate
	5	5 mL	5 mL/ min.
OUT:	Time	Output	State
	5.1	1	Pulse

Program Sheet

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

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305 Programming Sheet

File Number:	3	Method Name:	AutoPrep

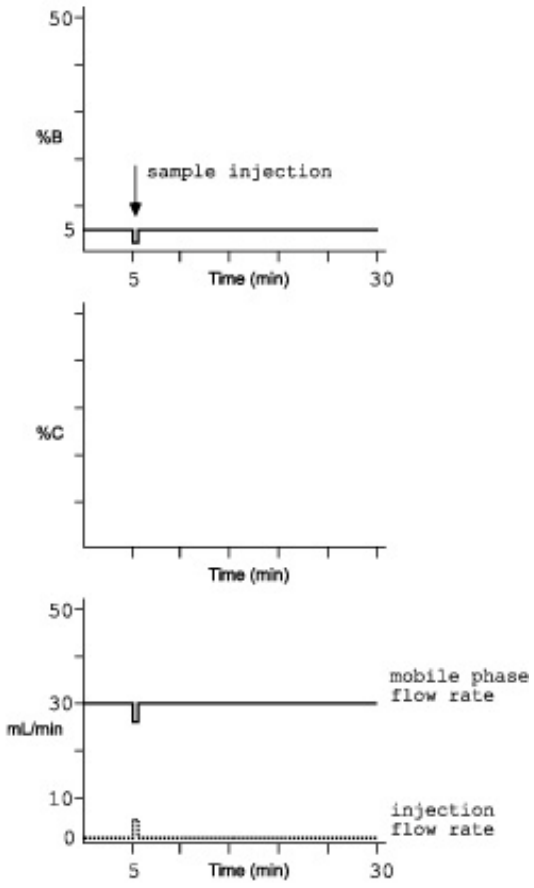
SETUP PARAMETERS

Pressure: High limit = 3000	Low limit = 0	Units: PSI				
Number of pumps = 3	Loops = 4	Link file = None				
Pump	**Model**	**Solvent**	**ID No.**	**Refill**	**Comp.**	**Headsize**
A	305	ACN	1	125	99	50
B	306	Water	2	125	46	50
C						
Inj.	306	Sample	4	125		10

I/O OPERATIONS

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

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	OUT	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			

Autopreparative Collection Controlled by 305 Pump
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Collecting Sharp Narrow Peaks K

With the FC 204 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.

Note: Detailed information on how a FC 204 Fraction Collector locates peaks using the peak detection algorithm can be found in ***Appendix H, Peak Detection Using Slope or Millivolt Level***.

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.

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In Figure 1, peak width and height are set as suggested in ***Appendix H, Peak Detection Using Slope or Millivolt Level***. With those settings, only half of the large peak at 4.4 minutes is collected.

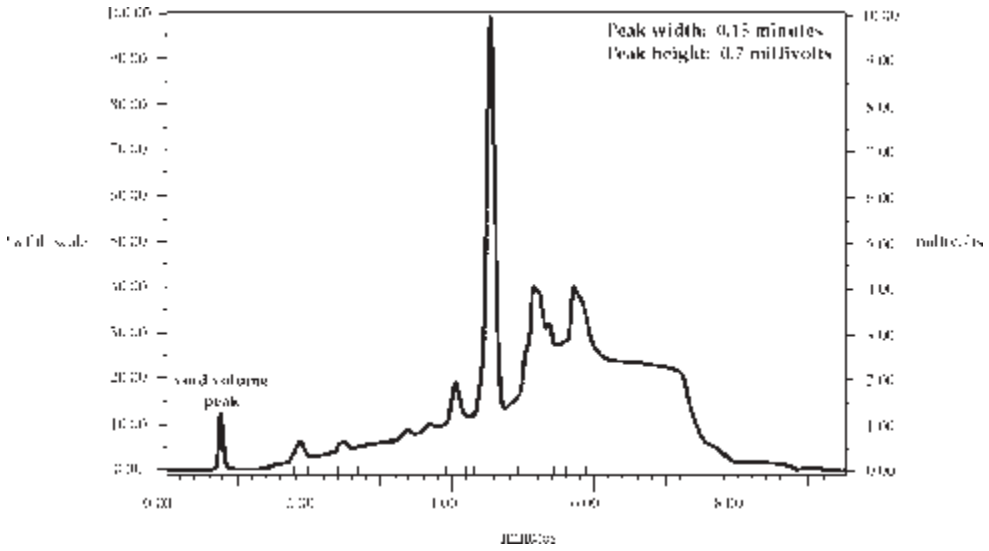


Figure 1

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.

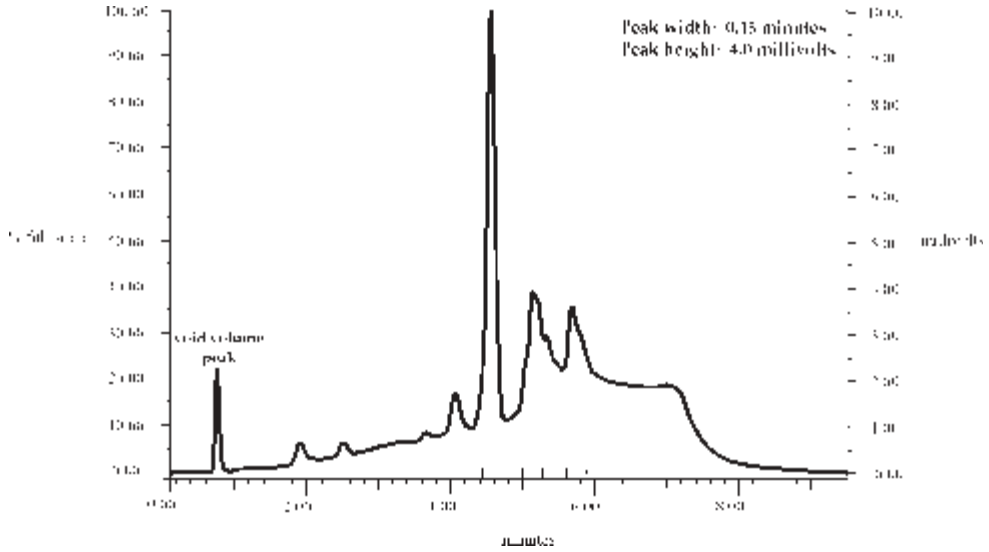


Figure 2

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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.

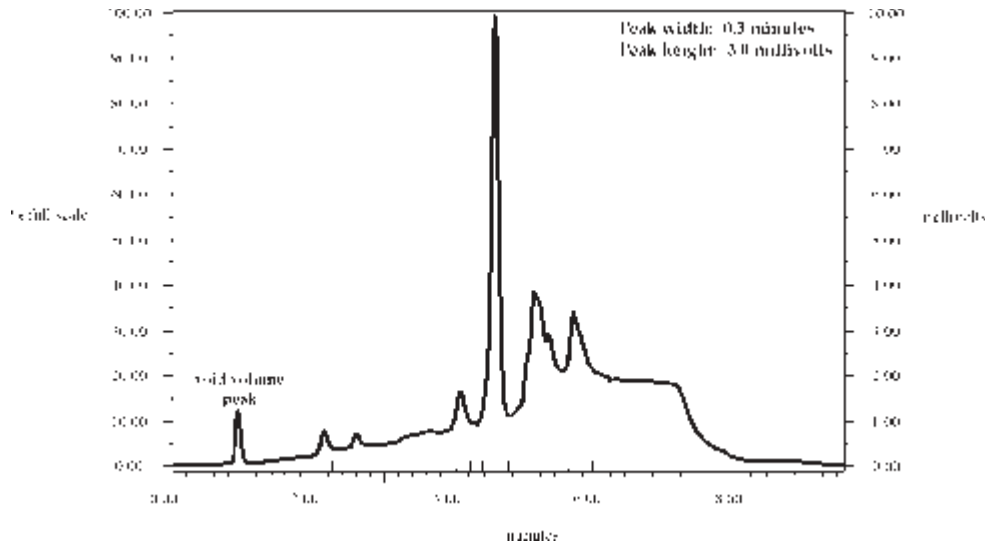


Figure 3

If your fraction collector is operating in a system controlled by Gilson UniPoint™ 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™ System Software User's Guide* for more information.

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Replacement Parts and Accessories L

Part number	*Description*
174732	Multiple column adapter. To collect fractions simultaneously from up to 9 columns. Up to 18 columns can be collected with two adapters.
1707305	Small bore dispense needle, 0.010" titanium, for Code 16 rack with 3-way valve kit
1727304	Large bore dispense needle kit, 1/ 8" OD, titanium, for collecting at flow rates greater than 100 mL/ minute with 3-way valve kit. Includes titanium large bore dispense needle and 1/ 4"-28 stainless steel fitting
1707303	Standard bore needle for 3-way valve
170734	3-way valve kit with 1.5 mm ID internal ports and 3.5 µL dead volume. Includes inlet and outlet tubing, connector and dispensing needle.
170735	FC 204 Prep Valve Assembly Package. 3-way valve kit, for flow rates up to 200 mL/ min.
1703549	1.5 mm ID inlet drop tubing assembly
1703548	0.8 mm ID inlet drop tubing assembly
1307302	Inlet tubing assembly for 3-way valve, 2 ft.
1307303	Drain tubing assembly for 3-way valve, 2 ft.
470343706	Drain tubing; 5/ 16" ID, per foot
709910206	2-conductor interconnect wire; 6 ft.

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6374022611	Pomona cable adapter
36078143	Shielded GSIOC cable; 30 in.
100731	Event cable
F1410050	Coupling for 1/ 4"-28 fittings; package of 5
1704744	Polypropylene tray
1704743	Tray platform for Code 21, 23, 23W, 24, 28, and 29 racks
638310512	10-pin terminal block connector
170354	Drop detector assembly, Valox. **Standard accessory.**
170355	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	Glass sleeve for drop detector; package of 6
171751	507 Power Module for remote power on/ off; 100/ 115V
6730054007	0.5 amp T-0.5 replacement fuse, Slo-Blo; 100/ 115V

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