

# **Waters Fraction Collector III**

## **Operator's Guide**

71500012102/Revision E

# Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Copyright © Waters Corporation 2015  
All rights reserved



# General Information

## Copyright notice

---

© 2015 WATERS CORPORATION. PRINTED IN THE UNITED STATES OF AMERICA AND IN IRELAND. ALL RIGHTS RESERVED. THIS DOCUMENT OR PARTS THEREOF MAY NOT BE REPRODUCED IN ANY FORM WITHOUT THE WRITTEN PERMISSION OF THE PUBLISHER.

The information in this document is subject to change without notice and should not be construed as a commitment by Waters Corporation. Waters Corporation assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Waters Corporation be liable for incidental or consequential damages in connection with, or arising from, its use. For the most recent revision of this document, consult the Waters Web site ([waters.com](http://waters.com)).

## Trademarks

---

Alliance, MassLynx, Micromass, "THE SCIENCE OF WHAT'S POSSIBLE.", and Waters are registered trademarks of Waters Corporation, and PrepLC is a trademark of Waters Corporation.

Gilson is a registered trademark of Gilson, Inc.

GeneVac is a registered trademark of Genevac Ltd.

VICTREX PEEK is a trademark of Victrex PLC.

Teflon is a registered trademark of E. I. du Pont de Nemours and Company.

Tygon is a registered trademark of Norton Company.

Other registered trademarks or trademarks are the sole property of their owners.

## Customer comments

---

Waters' Technical Communications organization invites you to report any errors that you encounter in this document or to suggest ideas for otherwise improving it. Help us better understand what you expect from our documentation so that we can continuously improve its accuracy and usability.

We seriously consider every customer comment we receive. You can reach us at [tech\\_comm@waters.com](mailto:tech_comm@waters.com).

## Contacting Waters

---

Contact Waters with enhancement requests or technical questions regarding the use, transportation, removal, or disposal of any Waters product. You can reach us via the Internet, telephone, or conventional mail.

### Waters contact information


Contacting medium	Information
Internet	The Waters Web site includes contact information for Waters locations worldwide. Visit <a href="http://www.waters.com">www.waters.com</a> .
Telephone and fax	From the USA or Canada, phone 800-252-4752, or fax 508-872-1990. For other locations worldwide, phone and fax numbers appear in the Waters Web site.
Conventional mail	Waters Corporation Global Support Services 34 Maple Street Milford, MA 01757 USA

## Safety considerations

---

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's standard operating procedures.

### Safety hazard symbol notice

Documentation needs to be consulted in all cases where the  symbol is used to find out the nature of the potential hazard and any actions which have to be taken.

### Considerations specific to the Waters Fraction Collector III

#### Power cord replacement hazard



**Warning:** To avoid electric shock, use the SVT-type power cord in the United States and HAR-type (or better) cord in Europe. The main power cord must be replaced only with one of adequate rating. For information regarding what cord to use in other countries, contact your local Waters distributor.

## Bottle placement prohibition



**Warning:** To avoid injury from electrical shock or fire, and damage to the equipment, do not place vessels containing liquid atop the workstation or ancillary equipment or otherwise expose those units to dripping or splashing liquids.



**Prohibited:** Do not place vessels containing liquid—such as solvent bottles—atop the workstation or ancillary equipment or otherwise expose those units to dripping or splashing liquids.

## FCC radiation emissions notice

Changes or modifications not expressly approved by the party responsible for compliance, could void the users authority to operate the equipment. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

## Electrical power safety notice

Do not position the instrument so that it is difficult to disconnect the power cord.

## Equipment misuse notice

If equipment is used in a manner not specified by its manufacturer, protections against personal injury inherent in the equipment's design can be rendered ineffective.












## Safety advisories

Consult [Appendix A](#) for a comprehensive list of warning advisories and notices.

# Operating this instrument

When operating this instrument, follow standard quality-control (QC) procedures and the guidelines presented in this section.

## Applicable symbols

Symbol	Definition
	Manufacturer
	Date of manufacture
	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
	Australia EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use
	Alternating current
	Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste. For compliance with the Waste Electrical and Electronic Equipment Directive (WEEE) 2012/19/EU, contact Waters Corporation for the correct disposal and recycling instructions.
	Serial number
	Part number catalog number

## Audience and purpose

This guide is intended for personnel who install, operate, and maintain the Waters Fraction Collector III.

## Intended use of the Waters Fraction Collector III

The Waters Fraction Collector III is intended for general laboratory use only. The Waters Fraction Collector III is not intended for use in diagnostic applications.

## Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards must include the entire range of QC samples, typical specimens, and atypical specimens.

## Quality control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. If sample trays are the same or very similar, vary the location of the QC samples in the trays. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

## EMC considerations

---

### Canada spectrum management emissions notice

This class A digital product apparatus complies with Canadian ICES-001.

Cet appareil numérique de la classe A est conforme à la norme NMB-001.

### ISM Classification: ISM Group 1 Class B

This classification has been assigned in accordance with IEC CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements.

Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment.

Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low voltage, power-supply network.

## EC authorized representative

---



Waters Corporation  
Stamford Avenue  
Altrincham Road  
Wilmslow SK9 4AX UK

Telephone: +44-161-946-2400  
Fax: +44-161-946-2480  
Contact: Quality manager



# Table of Contents

---

<b>General Information</b> .....	<b>iii</b>
Copyright notice .....	iii
Trademarks .....	iii
Customer comments .....	iii
Contacting Waters .....	iv
Safety considerations .....	iv
Safety hazard symbol notice.....	iv
Considerations specific to the Waters Fraction Collector III .....	iv
FCC radiation emissions notice.....	v
Electrical power safety notice.....	v
Equipment misuse notice .....	v
Safety advisories .....	v
Operating this instrument .....	vi
Applicable symbols.....	vi
Audience and purpose .....	vii
Intended use of the Waters Fraction Collector III.....	vii
Calibrating .....	vii
Quality control.....	vii
EMC considerations .....	vii
Canada spectrum management emissions notice.....	vii
ISM Classification: ISM Group 1 Class B .....	vii
EC authorized representative .....	viii
<b>1 Overview</b> .....	<b>15</b>
1.1 About the Fraction Collector III .....	15
1.1.1 Collection configurations.....	16
1.2 Fraction Collector III components .....	18
1.2.1 Standard components .....	19
1.2.2 Optional racks.....	20
1.3 Operating modes .....	24
<b>2 Installing the Waters Fraction Collector III</b> .....	<b>27</b>
2.1 Installation overview .....	27
2.1.1 Required materials.....	27

2.2	Site requirements .....	28
2.3	Unpacking .....	28
2.4	Making power connections .....	29
2.5	Making fluidic connections .....	30
2.5.1	Connecting the sample tubing .....	31
2.5.2	Connecting the drain tubing .....	33
2.5.3	Installing a 3-way valve.....	35
2.6	Making signal connections .....	38
2.6.1	Making I/O signal connections .....	38
2.7	Installing the standard test tube rack .....	40
2.7.1	Test tube supports .....	40
2.8	Installing optional racks .....	41
2.8.1	Installing the prep funnel and funnel rack .....	41
2.8.2	Installing the 28-mm vial rack.....	43
2.8.3	Installing the micro plate rack .....	43
2.8.4	Installing the carousel rack .....	44
2.8.5	Installing the 17-mm vial rack.....	44
2.8.6	Installing the Eppendorf tube rack .....	45
2.8.7	Installing the multi-purpose rack .....	45
<b>3</b>	<b>Preparation and operation .....</b>	<b>47</b>
3.1	Powering on the unit .....	47
3.1.1	Initializing the memory .....	48
3.2	LCD display and keypad overview .....	49
3.2.1	LCD display .....	49
3.2.2	Keypad functions.....	49
3.3	Preliminary setup .....	50
3.3.1	Setting conditions.....	51
3.3.2	Selecting the rack.....	53
3.3.3	Programming bottle positions.....	56
3.3.4	Dropper assembly movement.....	58
<b>4</b>	<b>Collecting Fractions Using Simple Modes .....</b>	<b>63</b>
4.1	Collecting using time .....	63
4.1.1	Setting TIME parameters .....	63
4.1.2	Running the program .....	65
4.1.3	Sample TIME mode program .....	67

4.2	Collecting using drop count .....	68
4.2.1	Setting DROP Parameters .....	68
4.2.2	Running the program .....	69
4.2.3	Sample DROP mode program .....	70
4.3	Collecting using signal input .....	71
4.3.1	Setting SIGNAL parameters .....	72
4.3.2	Running the program .....	73
4.3.3	Sample SIGNAL mode program .....	74
4.4	Collecting using volume .....	75
4.4.1	Setting VOLUME parameters .....	75
4.4.2	Running the program .....	76
4.4.3	Sample VOLUME mode program .....	78
<b>5</b>	<b>Collecting Fractions Using Advanced Modes .....</b>	<b>81</b>
5.1	Collecting using peak discrimination .....	81
5.1.1	Setting PEAK parameters.....	83
5.1.2	Running the program .....	88
5.1.3	Sample PEAK mode program.....	89
5.2	Collecting using time windows .....	90
5.2.1	Setting WINDOW parameters .....	90
5.2.2	Running the program .....	93
5.2.3	Sample WINDOW mode program .....	95
5.3	Collecting using a programmed sequence .....	96
5.3.1	Setting MANUAL parameters .....	97
5.3.2	Running the program .....	98
5.4	Collecting using sampling intervals .....	100
5.4.1	Setting SAMPLING parameters .....	100
5.4.2	Running the program .....	101
5.4.3	Sample SAMPLING mode program .....	103
5.5	Displaying the results of a run .....	104
5.5.1	Table display.....	104
5.5.2	Graph display.....	106
5.6	Using event markers to track peaks .....	107
<b>6</b>	<b>Maintenance .....</b>	<b>109</b>
6.1	Spare parts .....	109
6.1.1	<b>Contacting Waters technical service .....</b>	<b>109</b>

6.2	Safety and handling .....	109
6.3	Running the test mode diagnostics .....	110
6.3.1	Testing the dropper assembly movement .....	110
6.3.2	Testing the panel keys.....	111
6.3.3	Testing the 3-way valve.....	111
6.3.4	Testing the I/O terminal .....	112
6.3.5	Testing the chromatography signal.....	112
6.3.6	Testing the drop sensor .....	112
6.4	Troubleshooting .....	113
6.4.1	Basic troubleshooting steps.....	113
6.4.2	System troubleshooting.....	114
6.4.3	Signal troubleshooting .....	115
6.4.4	Collection troubleshooting.....	116
<b>A</b>	<b>Safety Advisories .....</b>	<b>119</b>
A.1	Warning symbols .....	119
A.1.1	Specific warnings .....	120
A.2	Notices .....	121
A.3	Bottles Prohibited symbol .....	122
A.4	Required protection .....	122
A.5	Warnings that apply to all Waters instruments and devices .....	123
A.6	Warnings that address the replacing of fuses .....	126
A.7	Electrical and handling symbols .....	127
A.7.1	Electrical symbols.....	127
A.7.2	Handling symbols.....	128
<b>B</b>	<b>Specifications .....</b>	<b>131</b>
<b>C</b>	<b>Spare Parts and Accessories .....</b>	<b>133</b>
C.1	Fraction Collector III spare parts .....	133
C.2	Fraction Collector III accessories .....	133
<b>D</b>	<b>Chemical Compatibility .....</b>	<b>135</b>
D.1	Introduction .....	135
D.1.1	Clean solvents .....	135
D.1.2	Solvent quality.....	135

D.1.3	Preparation checklist .....	135
D.1.4	Water .....	135
D.1.5	Buffers .....	136
D.1.6	THF.....	136
D.2	Component chemical compatibility .....	136



# 1 Overview

This chapter introduces you to the Waters Fraction Collector III (WFC III). It describes the Fraction Collector III components and operating modes, and lists available optional racks.

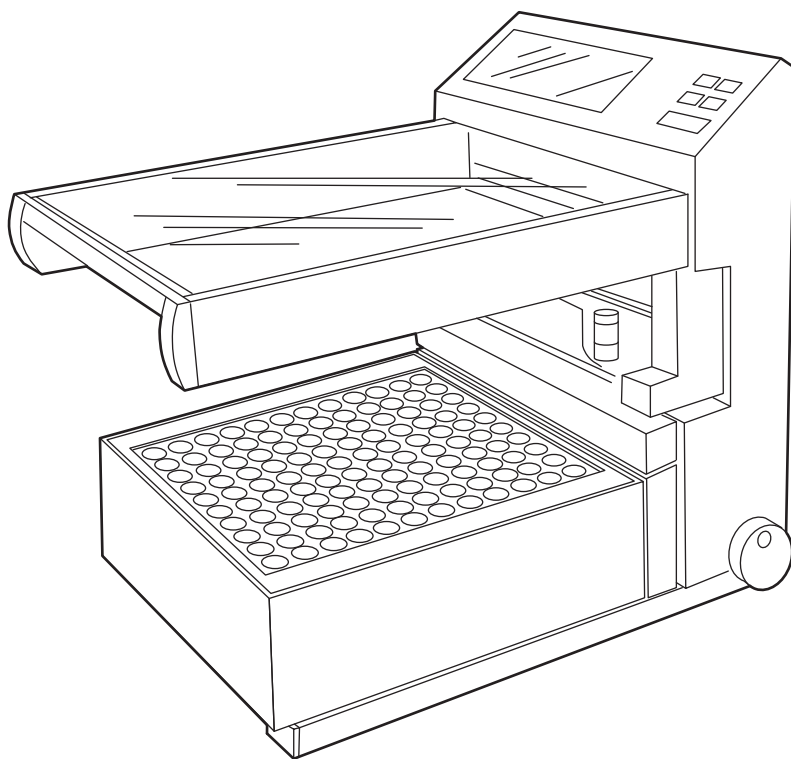
Refer to [Appendix B](#) for WFC III specifications. [Appendix D](#) provides information on the compatibility of the WFC III's components with a variety of chemicals.

## 1.1 About the Fraction Collector III

---

The Waters Fraction Collector III is a programmable, easy-to-use, stand-alone fraction collection module. The WFC III is compatible with the Waters Autopurification MS and UV systems using FractionLynx and any Waters PrepLC™ system, specifically the Delta 600, 4000 Series, and the PrepLC 2000, as well as other Waters and non-Waters HPLC modules and systems. When combined with a Waters PrepLC system, the WFC III provides a flexible PrepLC system package that is easy to use. A range of optional accessories that allow the unit to work with a variety of sample collection devices is available for the WFC III. See [Appendix C](#) for a list of optional accessories.

**Figure 1-1: Waters Fraction Collector III**



### **1.1.1 Collection configurations**

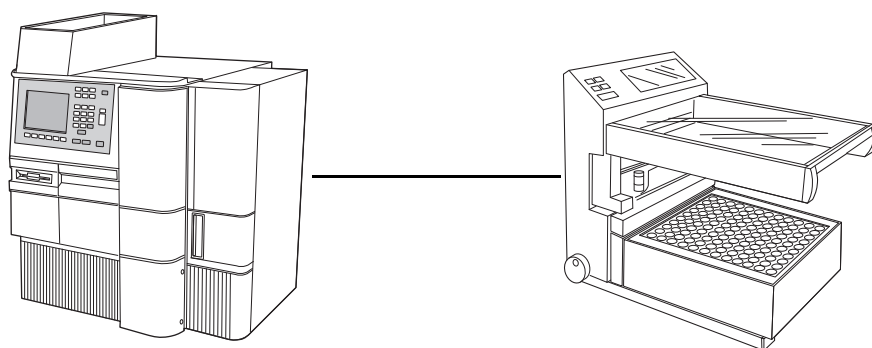
The WFC III can be used in three collection configurations:

- Single WFC III unit for collection of fractions
- Dual WFC III units for collection of peak fractions and volume between peaks
- Multiple WFC III units for high throughput autopurification

#### **1.1.1.1 Single WFC III unit for HPLC**

You can use a single WFC III to collect fractions from an HPLC or LC/MS system.

**Figure 1-2: Single WFC III configuration**

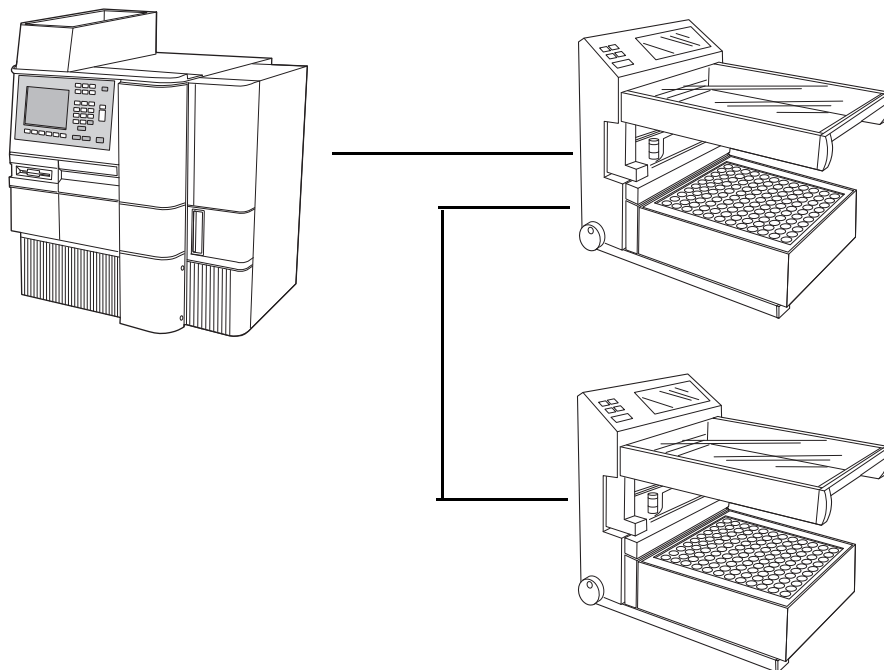




### 1.1.1.2 Dual WFC III units for MassLynx

You can use dual WFC III units to collect peak fractions and volume between peaks, which would normally be diverted to waste. This configuration is supported only in systems controlled by MassLynx® software. Dual WFC III units must be connected serially.

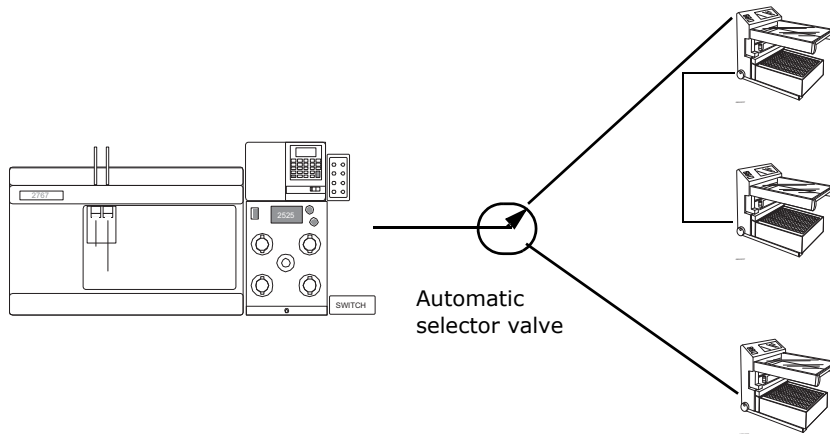
**Figure 1–3: Dual WFC III configuration**



### 1.1.1.3 Multiple WFC III units for high throughput

You can use multiple WFC III units and the MassLynx software to collect fractions from high throughput autopurification. This configuration requires the use of an automatic selector valve connected to up to eight WFC III units or to eight pairs of WFC III units connected as shown for dual operation. The maximum number of WFC III units that can be connected to a system is 16.

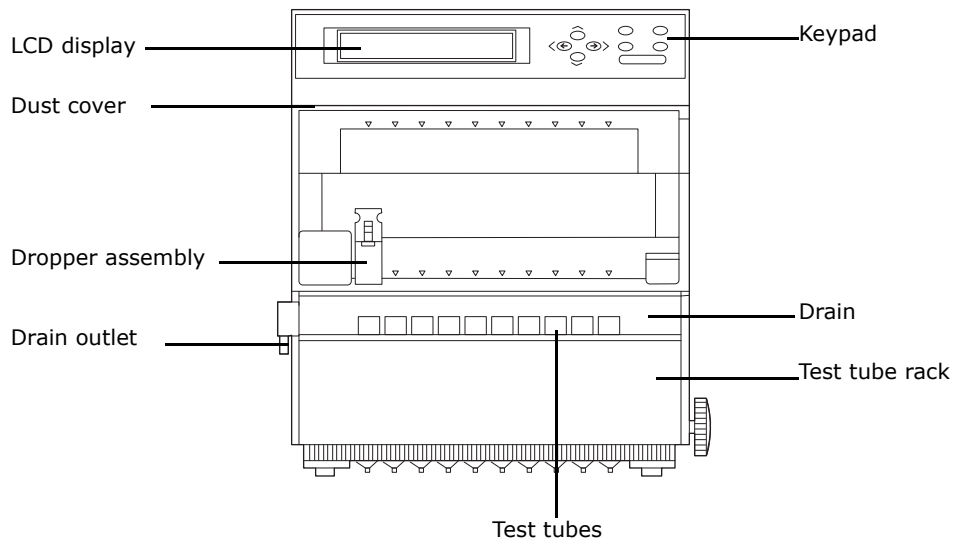
**Figure 1-4: Multiple WFC III configuration**



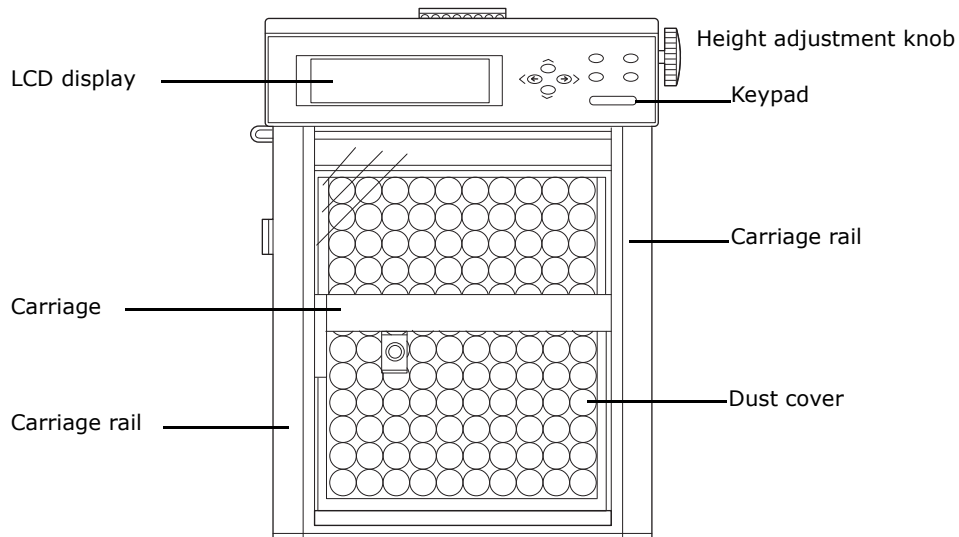
## 1.2 Fraction Collector III components

Before you install the WFC III, familiarize yourself with its components.

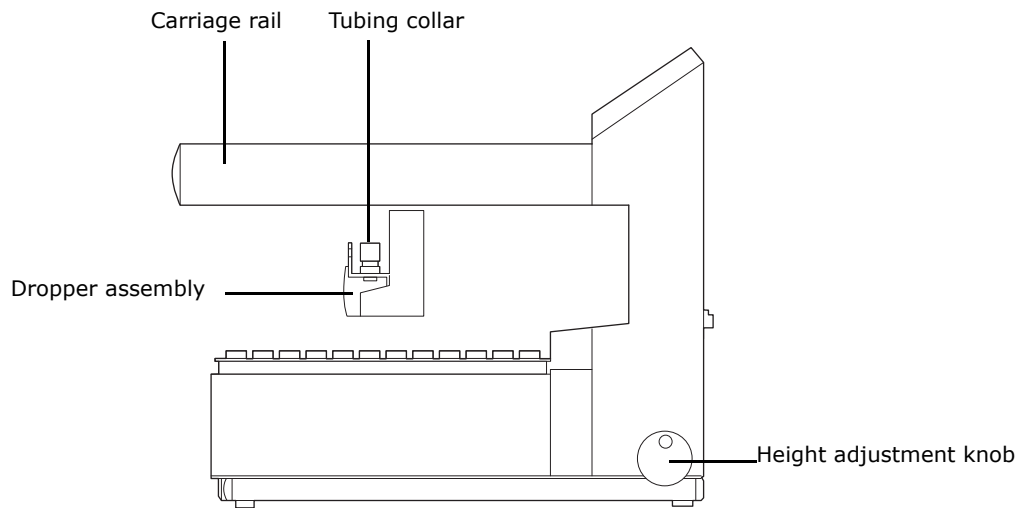
**Figure 1-5: Fraction Collector III – front view**



**Figure 1-6: Fraction Collector III – top view**



**Figure 1-7: Fraction Collector III – side view**



### 1.2.1 Standard components

The components of the WFC III are listed below:

- Fraction Collector III body with drain
- Standard test tube rack
- Dust cover
- Drip tray

- 3-way valve (not installed upon shipment)
- I/O terminal connector, attached to the rear of the WFC III body
- Connector cable cores: GRFC-10, GRFC-8 and GRFC-9
- Teflon sample tubing, 2.0 mm OD × 1.0 mm ID × 1 m
- Silicone drain tubing, 8.0 mm OD × 5.0 mm ID × 0.5 m
- Tube suspenders (2)
- LC connection kit
- Power cord
- *Waters Fraction Collector III Operator's Guide*

The 3-way valve diverts the solvent flow to a waste vessel as the dropper assembly moves between collection vessel positions. Flow may also be directed to a waste vessel during nonwindow portions of a collection procedure.

A low residual 3-way valve is available as an option. The flow rate of the low residual valve is approximately 40% of the flow of the standard valve. For detailed 3-way valve specifications, see the [table titled "3-way valve specifications" on page 132](#).

**Tip:** If the WFC III is to be used as part of a system, you should attach the cable cores to your cable connections.

## 1.2.2 Optional racks

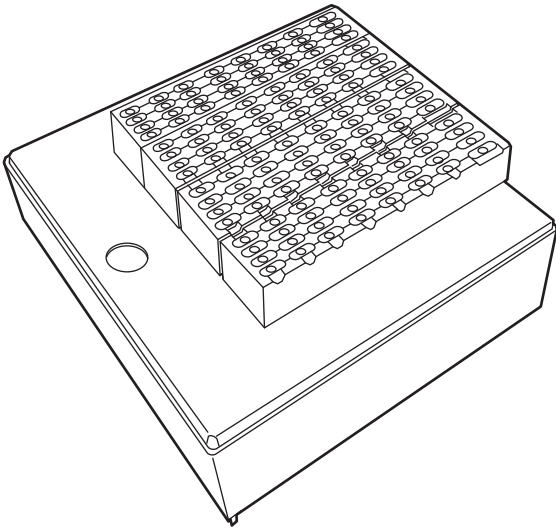
The optional racks available for the WFC III are described in this section. To learn how to install these racks, see ["Installing optional racks" on page 41](#).

### 1.2.2.1 Prep funnel and funnel rack

The optional prep funnels and rack replace the standard tube rack and are used to collect larger volume fractions in up to 128 large bottles or flasks. Up to four prep funnels (32 fractions each) can be used with the funnel rack assembly, along with the optional tabletop stand.

**Note:** The funnel rack is not approved by CSA certification.

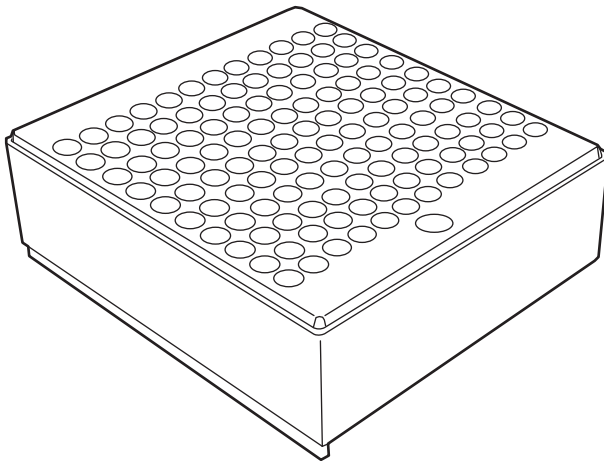
**Figure 1-8: Prep funnel rack**



### **1.2.2.2 17-mm vial rack**

The optional 17-mm vial rack replaces the standard tube rack. It allows collection of samples into 17-mm OD scintillation vials. The rack can hold 120 vials.

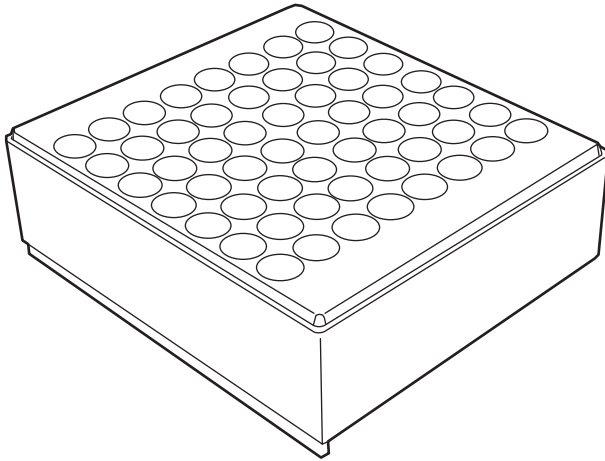
**Figure 1-9: 17-mm vial rack**



### **1.2.2.3 28-mm vial rack**

The optional 28-mm vial rack replaces the standard tube rack. It allows collection of samples into 28-mm OD scintillation vials. The rack can hold 56 vials.

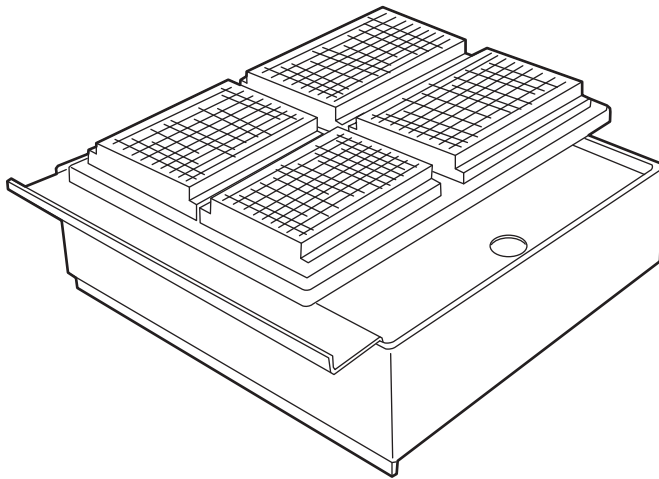
**Figure 1–10: 28-mm vial rack**



#### **1.2.2.4 Micro plate rack**

The optional micro plate rack replaces the standard tube rack. It allows collection of samples into four 96-well micro titer plates (standard or deep-well). The plates are not included with the rack. You should use this rack with the extension nozzle.

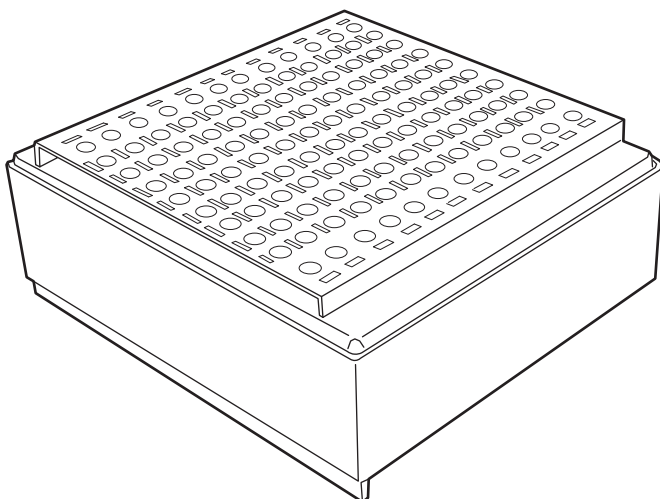
**Figure 1–11: Micro plate rack**



#### **1.2.2.5 Eppendorf tube rack**

The optional Eppendorf tube rack replaces the standard tube rack. It allows collection of samples into 1.5-mL Eppendorf tubes. The rack can hold 120 tubes.

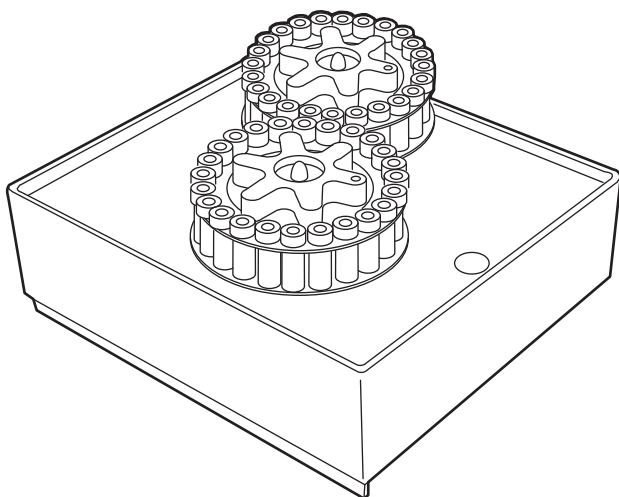
**Figure 1–12: Eppendorf tube rack**



### **1.2.2.6 Carousel rack**

The optional carousel rack replaces the standard tube rack. It allows for collection of samples into 2-mL vials in Alliance® carousels. The rack can accommodate one or two 24-vial carousels. The carousels are not included with the rack.

**Figure 1–13: Carousel rack**



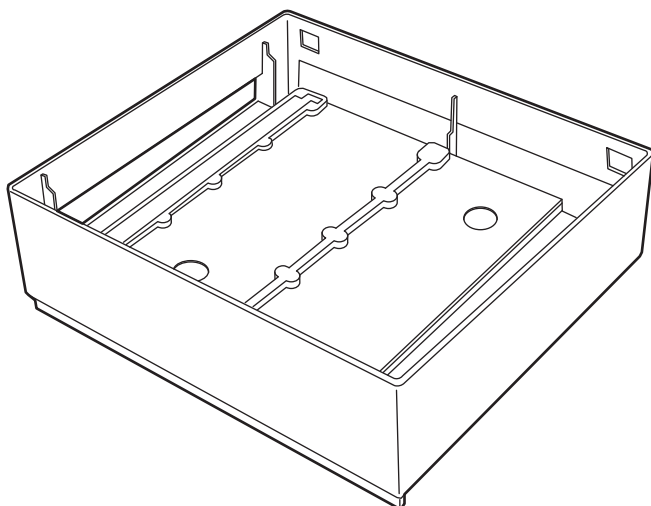
### **1.2.2.7 Multi-purpose rack**

The optional multi-purpose rack replaces the standard tube rack. It accepts a variety of racks, including:

- One Waters 2700 Sample Manager rack
- Up to two Gilson FC 204 Fraction Collector racks
- Up to eight GeneVac test tube holders (eight or twelve tubes per holder)

- A combination of one Gilson FC 204 Fraction Collector rack and up to four GeneVac test tube holders

**Figure 1–14: Multi-purpose rack**



## 1.3 Operating modes

---

The WFC III offers several operating modes. The individual modes are grouped into Utility, Simple, and Advanced categories by function. The [table titled "WFC III modes" on page 24](#) provides an overview of the available modes. For full details of the individual modes, see [Chapter 3](#), through [Chapter 6](#).

**Table 1–1: WFC III modes**

Mode	Collection method	Notes
<b>Utility modes</b>		
DISPLAY RESULT	Displays the results of the previous collection run as a table or graph.	Graph display is only available for PEAK, WINDOW, and MANUAL collection runs.
CONDITIONS	Sets the instrument display, operation, and input/output conditions.	N/A
RACK SELECTION	Sets the instrument for the type of rack used in a collection run.	Selection is displayed in the upper-right corner of the LCD.
BOTTLE POSITION	Sets instrument to collect samples into preset matrix or custom arrangement of bottles.	N/A



**Table 1-1: WFC III modes (continued)**

<b>Mode</b>	<b>Collection method</b>	<b>Notes</b>
TEST	Runs diagnostic tests on instrument.	Access this mode by selecting <b>CONDITIONS</b> and pressing <b>END</b> .
<b>Simple modes</b>		
TIME	Time per collection vessel.	Can use the multi-sample option.
DROP	Drop per collection vessel.	Can use the multi-sample option.
SIGNAL	Count per collection vessel using an external signal.	Can use the multi-sample option.
VOLUME	Volume per collection vessel.	Can use the multi-sample option.

**Table 1-1: WFC III modes (continued)**

<b>Mode</b>	<b>Collection method</b>	<b>Notes</b>
<b>Advanced modes</b>		
PEAK	Uses chromatogram input to discriminate peaks by peak number, absolute value, slope angle, or absolute value and slope angle.	Can use the multi-sample option.
WINDOW	Within preset time windows, collects only the samples complying with specified parameters.	Can use the multi-sample option. Can set PEAK collecting parameters within WINDOW.
MANUAL	Using manual input of a sequence of commands (TEACHING function) can replay the program (PLAYBACK function) to automatically collect samples.	N/A
SAMPLING	Takes samples from the column/detector effluent at specified time intervals. Can collect repeated samples into the same collection vessel.	Sometimes referred to as "pooling of fractions".

# 2 Installing the Waters Fraction Collector III

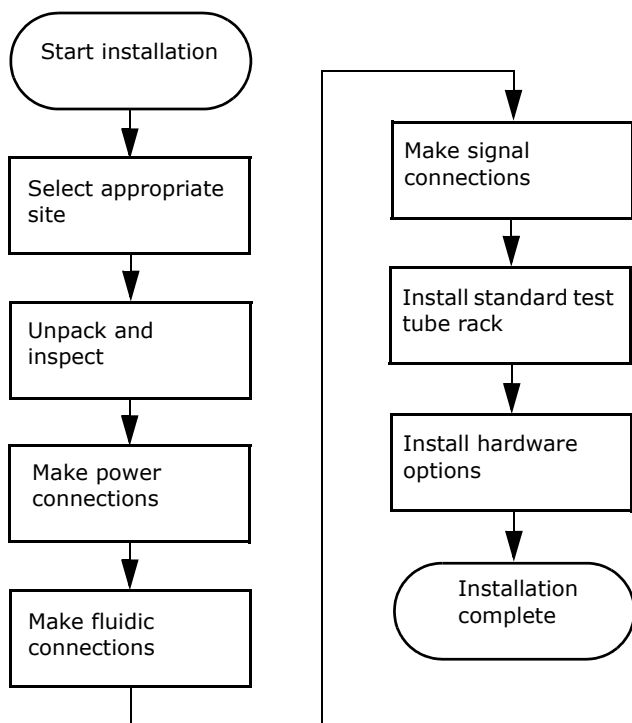
This chapter describes how to make power, fluidic, and signal connections to the Waters Fraction Collector III. It also describes how to add hardware options.

## 2.1 Installation overview

---

The figure below shows the major steps in installing the Fraction Collector III.

**Figure 2–1: Installing the Fraction Collector III**



### 2.1.1 Required materials

You need the following materials to install the WFC III:

- Waters PEEK tubing cutter (part number WAT031795) or similar tool to cut tubing
- Wire insulation strippers to prepare wires for the I/O terminal

- Small screwdriver to secure wires to the I/O terminal

After you install the WFC III, proceed to [Chapter 3](#).

## 2.2 Site requirements

---

Install the WFC III at a level, stable bench that meets the specifications indicated in the table below. The unit should not be exposed to water, corrosive gases, extreme temperatures, direct sunlight, or infrared rays.

- !** **Notice:** The front and top housing of the WFC III are constructed of ABS plastic and can be damaged by solvents. Do not allow solvent to contact these parts. Always keep the area surrounding the WFC III clean and wipe up solvent spills immediately.

**Table 2–1: Installation site requirements**

Factor	Requirements
Temperature	4 to 40 °C (39 to 104 °F)
Relative humidity	20 to 85%, noncondensing
Bench space	W 12 1/4 in. × D 16 1/4 in. × H 14 1/4 in. (W 311 mm × D 413 mm × H 362 mm)
Power	0.23 A at 100 V, 0.2 A at 115 V, 0.15 A at 240 V 50 to 60 Hz

## 2.3 Unpacking

---

The WFC III is shipped in a single carton. Save the carton in case you decide to transport or ship the unit in the future. Accessories such as rack options are shipped in separate cartons.

- !** **Notice:** When unpacking or moving the WFC III, be careful not to lift the unit by the carriage rails. Be sure to always lift the unit with one hand under the base.

### To unpack the WFC III:

1. Open the carton and remove the Startup Kit and the packing material.
2. Check the contents of the carton against the packing slip to confirm that all items are included.
3. Lift the WFC III from the carton and place it at the site you have chosen for installation.

4. Inspect all items for damage. Immediately report any shipping damage to both the shipping company and your Waters representative. Contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary, or call Waters corporate headquarters in Milford, Massachusetts (U.S.).

Once you have unpacked the WFC III, continue the installation by following the procedures in ["Making fluidic connections" on page 30](#).

## 2.4 Making power connections

---

The WFC III can accommodate power sources from 100 to 240 Vac (50 to 60 Hz).

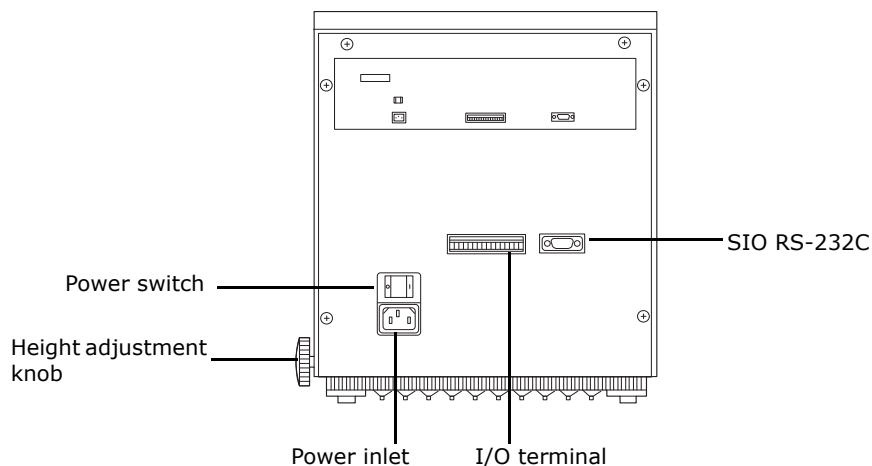
**Requirement:** You must use a power cord that is compatible with your local region's electrical source.

### To connect the WFC III to the power source:

1. Plug the female end of the power cord into the power inlet at the rear of the WFC III.
2. Plug the male end of the power cord into an appropriate 100 to 240 V (50 to 60 Hz) grounded power outlet.

Once you have made the power connections, continue the installation by following the procedures in ["Making fluidic connections" on page 30](#).

**Figure 2-2: Rear panel of the WFC III**



! **Notice:** The unit should be unplugged if it will not be used for a long time. However, if the unit is kept in an area of low temperature, the power cord should be plugged in at all times to prevent condensation within the unit.

## 2.5 Making fluidic connections

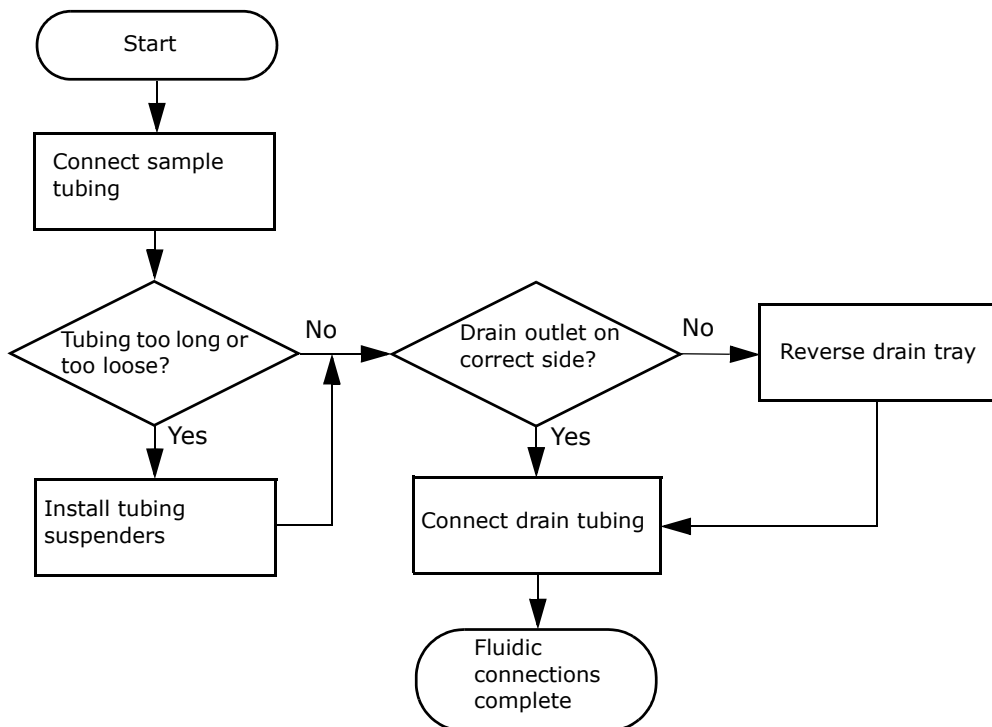
This section includes instructions for making fluidic connections to the WFC III. There are several types of fluidic connections you need to make:

- Sample tubing
- Drain tray
- Drip tray (optional)
- Drip tray drain tube plug
- 3-way valve (optional)

You can use the tubing provided with the unit or tubing purchased separately to make the fluidic connections.

The figure below summarizes the major steps in making fluidic connections to the WFC III. Once you have made all the necessary fluidic connections, continue the installation by following the procedures in ["Making signal connections" on page 38](#).

**Figure 2-3: Steps in making fluidic connections**



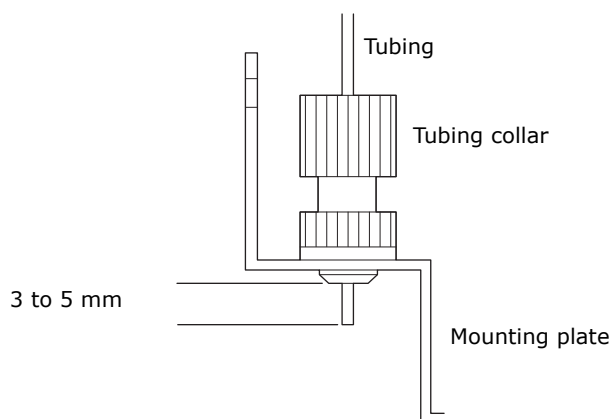
## 2.5.1 Connecting the sample tubing

The sample tubing carries liquid from the chromatography system to the WFC III. The tubing passes through the dust cover and is secured to the dropper assembly by the tubing collar.

### To install the sample tubing:

1. Estimate the tubing length needed between the chromatography system and the WFC III. The tubing should be long enough to allow the carriage unrestricted movement.
2. Cut the tube at a 90° angle to ensure proper drop formation. Use of a Waters PEEK tubing cutter (part number WAT031795) is recommended.
3. Pass one end of the tube through the slot in the left side of the dust cover.
4. Loosen the tubing collar on the dropper assembly and insert the tubing until it protrudes 3 to 5 mm from the bottom of the mounting plate.

**Figure 2-4: Installing the sample tubing on the dropper assembly**



5. Tighten the tubing collar so that the tubing is held firmly in place. Be sure to hand tighten only. Overtightening can strip the threads or otherwise stress the connections.
6. Connect the other end of the tube to your chromatography system.

**Tip:** To learn how to attach sample tubing to the optional 3-way valve, see ["Installing optional racks" on page 41](#).

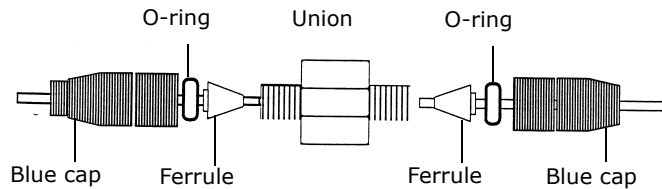
### 2.5.1.1 Sample tubing connection using alternative tubing sizes

While the tubing collar (tube joint) was designed to be used with 2.0-mm OD tubing, it can accommodate tubing with a larger OD. If tubing larger than 2.0-mm OD is used, join a segment of the tubing to the standard 2.0-mm OD tubing between the dropper assembly and the chromatography system.

If your tubing is less than 2.0-mm OD, you can increase the outer diameter of the tubing by wrapping a piece of tape around the tubing where it passes through the tubing collar.

If you are using stainless steel tubing, the tubing must be connected to a section of Teflon tubing before passing through the dust cover. Use the LC Connection Kit (part number WAT037048) to connect the stainless steel tubing to the Teflon tubing. The figure below shows the components of the LC Connection Kit.

**Figure 2-5: LC connection kit components**



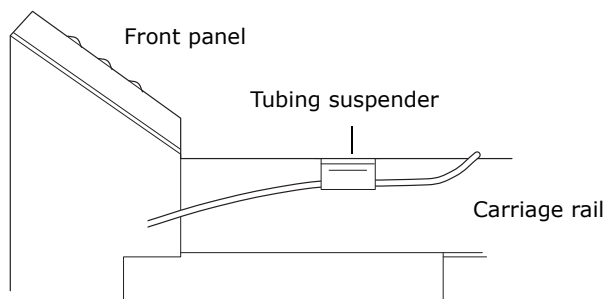
### 2.5.1.2 Installing tubing suspenders

If the sample tubing is too long or loose, it may become entangled on the dropper assembly or carriage. To avoid this difficulty, tubing suspenders are provided. These can be used to adjust the slack in the tubing, and can be attached as shown in the [figure "Placement of tubing suspender – side view" on page 32](#) and the [figure "Placement of tubing suspender – top view" on page 33](#).

#### To install tubing suspenders:

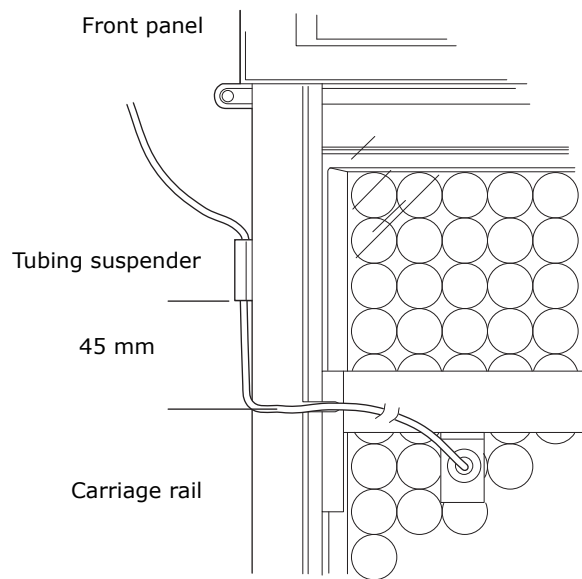
1. Install the sample tubing as described in the previous section.
2. Determine the optimal location for the tubing suspender.
3. Remove the backing from the adhesive strip on the tubing suspender.
4. Firmly press the tubing suspender onto the body of the WFC III at the desired location.
5. Slide the sample tubing into the tubing suspender.

**Figure 2-6: Placement of tubing suspender – side view**





**Figure 2-7: Placement of tubing suspender – top view**



## 2.5.2 Connecting the drain tubing

The drain tubing carries waste from the drain tray or the drip tray to a collection vessel.

**Tip:** When using drain tubing with either the drain tray or the drip tray, solvent may collect in the tubing and act as a trap preventing air from passing through the tube. If this occurs, the tray may fill up and overflow. To ensure fluid drains properly, make sure the tube is routed straight down into the tray and that the tube is not submerged in liquid.

### 2.5.2.1 Drain tray

The drain tray may be installed so the outlet is on either side of the WFC III.

#### To reverse the drain outlet position:

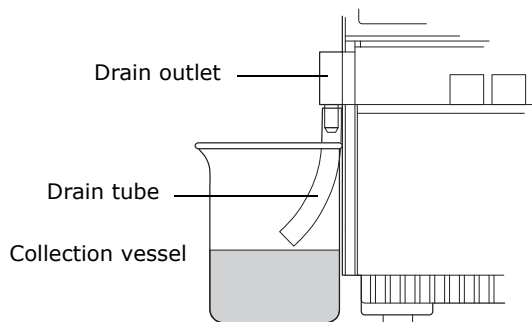
1. Remove the collection rack from the unit.
2. Grasp the top edge of the drain tray at both ends.
3. Pull forward firmly to disengage the drain tray. You may need to use a small screwdriver to gently pry the drain tray.
4. Reverse the drain tray so that the outlet is on the opposite side of the unit.
5. Align the drain tray feet with the slots.
6. Snap the drain tray into position.
7. Replace the collection rack.

Once the drain outlet is in the desired location you can connect the drain tubing.

**To connect the drain tubing to the drain tray:**

1. Push one end of the 8.0-mm OD drain tubing onto the WFC III drain outlet.
2. Place the other end of the tubing in a beaker or other collection vessel. Use a collection vessel that is less than 3 1/8 inches (8 cm) tall. If you need to use a larger collection vessel, place it at a level lower than the WFC III, such as on the floor or a cart.

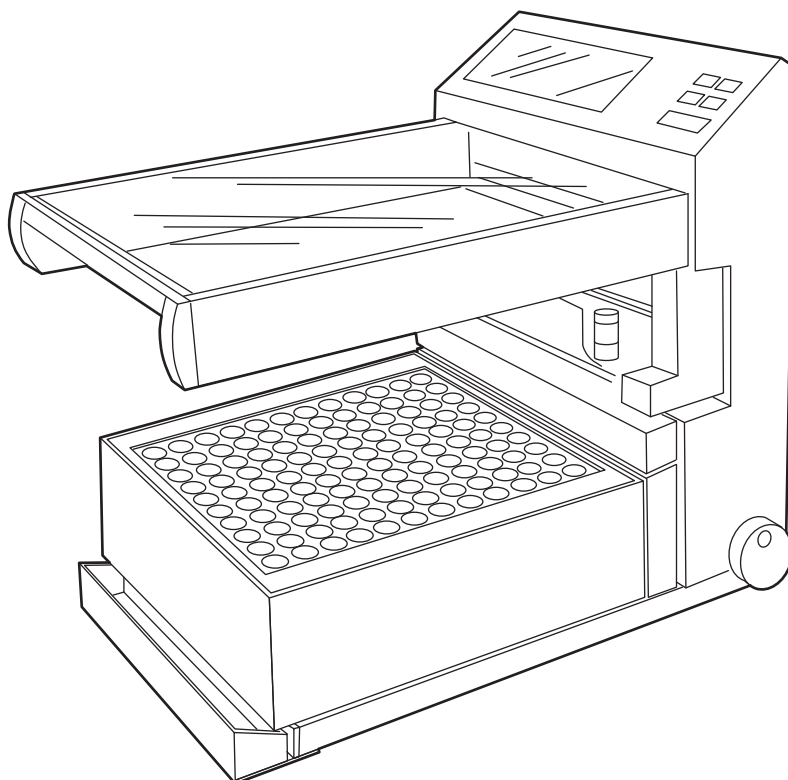
**Figure 2-8: Drain tubing installation**



### 2.5.2.2 Drip tray

The optional drip tray fits on the front of the WFC III. Use the drip tray if the arm will drain fluid past the rack. The drip tray is required only for stand-alone installations. If the WFC III is housed in the condo rack or uses the tabletop prep funnel rack, the drip tray is not needed.

**Figure 2–9: WFC III with drip tray installed**



**To install the drip tray:**

1. Lift the front of the unit slightly.
2. Slide the flat edge of the drip tray under the front of the unit.
3. Align the holes in the drip tray base with the rubber feet on the rails.
4. Gently set the WFC III down so that the rubber feet sit in the holes on the drip tray base.

**To connect the drain tubing to the drip tray:**

1. Push one end of the 8.0-mm OD drain tubing onto the drip tray drain outlet.
2. Place the other end of the tubing in a collection vessel. The vessel should be at a level lower than the WFC III, such as on the floor or a cart.

**Tip:** To use the drip tray as a collection reservoir, use the optional drain tube plug to close off the end of the drip tray.

### **2.5.3 Installing a 3-way valve**

You can install the 3-way valve (standard or low-residual) to divert the flow of solvent to a waste vessel as the dropper assembly moves between collection vessel positions. Flow may also be directed to a waste vessel during nonwindow portions of a collection procedure.

You install the 3-way valve in place of the standard dropper assembly. The installation procedure for both models of the valve (standard or low-residual) is identical.

The following materials are provided with the 3-way valve:

- Valve body
- Male connectors (2)
- Sample nozzle
- Allen wrench
- Seal adapter
- Flanged Teflon tubing



**Warning:** To avoid risk of serious injury from electric shock, be sure to unplug the power cord before proceeding with the 3-way valve installation.



**Notice:** Do not use excessive force on any of the wire or tubing connections.

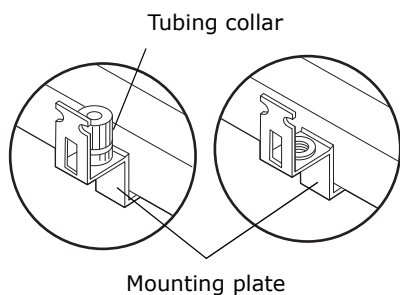
### To install the 3-way valve:

1. Power off and unplug the WFC III.
2. Remove the collection rack.
3. Gently slide the carriage toward the front of the unit.

**Tip:** Hold only the upper-right portion of the carriage to move it back and forth. Do not hold or push the carriage by the left edge.

4. Remove both sections of the tubing collar from the mounting plate of the dropper assembly. Unscrew the top portion first, then unscrew the lower portion. Do not use pliers as they may damage the plastic tubing collar parts.

### Figure 2–10: Removing the tubing collar from the mounting plate



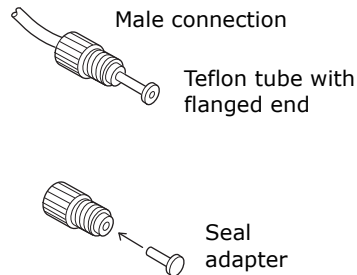
**Notice:** To avoid problems with backpressure, the drain tubing from the NO (normally open) side of the 3-way valve must have an ID greater than 0.5 mm and a length less than 1 meter.

5. Connect Teflon tubing with a flanged end (supplied with the valve) to the 3-way valve using the male connectors. The chromatography system should connect to

the COM port on the side of the valve, and the drain tubing should connect to the NO (normally open) port on the top of the valve.

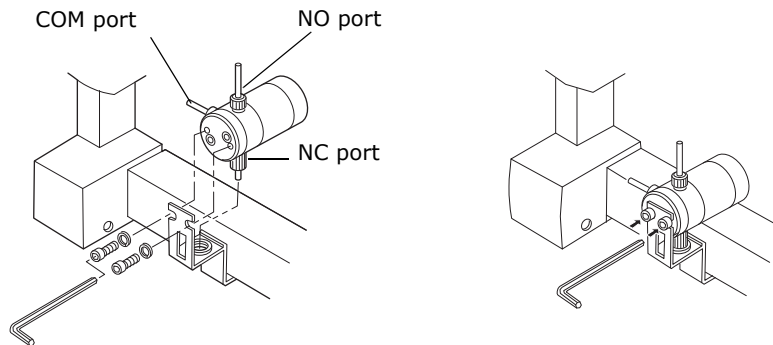
A seal adapter can be inserted into one of the male connectors as a plug to close the NO (waste) side of the 3-way valve.

**Figure 2-11: Connecting tubing using male connectors**



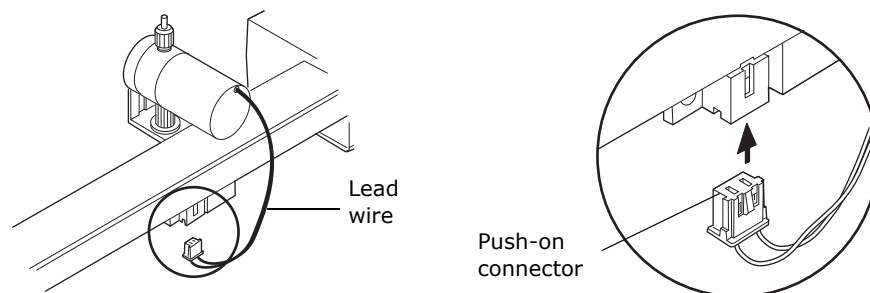
6. Remove the two Allen screws and their washers from the valve body using the supplied Allen wrench. Insert the sample dropper (NC, normally closed) on the 3-way valve into the hole in the metal mounting plate from which the tubing collar was removed. Thread the Allen screws through the notches on each side of the plate and tighten them into the valve body.

**Figure 2-12: Installing the 3-way valve on the mounting plate**



7. Insert the lead wire connector into the outlet on the underside of the dropper assembly by pushing upward. Adjust the lead wire so it does not make contact with the collection vessels at any time.

**Figure 2-13: Connecting the lead wire**



8. Pass the tubing attached to the 3-way valve through the dust cover. Connect the tubing from the COM port to the chromatography system. Run the tubing from the NO port to a waste collection vessel.
9. Plug in, then power on the WFC III. The dropper assembly moves to the ready position. Allow fluid to flow through the tubing and check for leaks at all connections.

**!** **Notice:** Fluids containing any particulate matter may damage the 3-way valve. If particulate-containing solvent may have been used or if the valve has not been used for a long time, flush out the valve with a clean solvent.

## 2.6 Making signal connections

---

The rear panel of the WFC III (see the [figure "Rear panel of the WFC III" on page 29](#)) provides a connection terminal for operation with external devices. This section describes the input/output (I/O) signal connections that you can make from the terminal block.

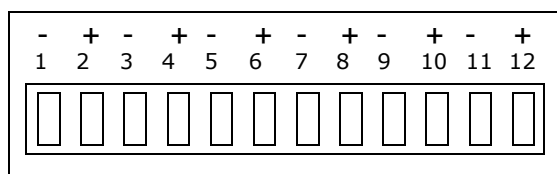
### 2.6.1 Making I/O signal connections

Your Waters Fraction Collector III communicates with your HPLC system through the I/O terminal. The I/O terminal is a two-part (male/female) connection. The female connector is permanently mounted on the rear panel of the Fraction Collector III. The I/O connector (male) snaps into the female terminal. Input and output leads are connected to the male portion of the terminal.

#### To make the I/O signal connections:

1. Select a suitable signal cable (supplied with the LC system module) and cut it to the appropriate length. The signal cables should be as short as possible to minimize interference.
2. Using a wire insulation stripper, remove approximately 5 mm of insulation from the end of each signal cable wire.
3. Remove the male connector from the rear panel of the WFC III by pulling firmly on the orange body of the I/O terminal.
4. Review the I/O terminal diagram below. (This diagram also appears on the rear panel of the unit.) Each numbered slot on the block diagram corresponds to the respective numbered pin.

**Figure 2-14: I/O terminal connector**



5. Look at the terminal block and locate the pin you want to connect (refer to the [table titled "Signal connections" on page 39](#) for the signal description for each pin). Positive connections are made to even-numbered terminals, negative connections are made to odd-numbered terminals.
6. Loosen the screw above the corresponding pin slot. Insert the wire lead all the way into the slot.
7. Tighten the screw to secure the wire lead and make contact.
8. After all connections have been made, reinsert the male connector (screws should be facing up) into the female connector.
9. Attach the GRFC-10 connector core to the I/O terminal connection cord. Make sure the distance between the core and the terminal connector is less than 10 cm.
10. Attach the GRFC-8 connector core to the RS-232 Serial Cable. Make sure the distance between the core and the rear panel terminal connector is less than 10 cm.
11. Attach the GRFC-9 connector core to the Power Cable. Make sure the distance between the core and the rear panel terminal connector is less than 10 cm.

The output signal of a mechanical contact such as a switch or relay can be used to provide input to the EXT START, EXT END, and EXT COUNT terminals. To prevent electrical interference, do not connect these output signals to other devices.

**Table 2–2: Signal connections**

Pin	Signal	Description
1(-)/2(+)	SIGNAL	Connect to the output from the detector. Use the CONDITIONS mode to select a signal level compatible with the detector output.
3(-)/4(+)	EXT START	Connect to an external start signal (e.g., pump controller).
5(-)/6(+)	EXT END	Connect end signal to pause fractionating.
7(-)/8(+)	EXT COUNT	Connect count signal for fractionating.
9(-)/10(+)	EVENT MARKER (1)	Can be connected to the input terminal of a recorder. Can be used to turn the pump off after a run.
11(-)/12(+)	EVENT MARKER (2)	ON status except during dropper movement after start. This can be used to turn pump off during dropper movement to prevent back pressure in the chromatography system.

**Tip:** If a hardware trigger is required by a timed event out (S1-S4) of a controller, a signal cable (part number 62031) may be required. To use the signal cable, connect the short black ground to terminal on the WFC and the white positive to S4 on the WFC. Connect the long lead black negative to S1-S4 and the white to +12V on the controller.

The table below shows the I/O terminal connector specifications.

**Table 2–3: I/O terminal connector specifications**

Parameter	Value
Maximum terminal voltage	60 Vdc
Maximum terminal current	0.3 A
Maximum switch wattage	10 W
Minimum resistance capacity	100 mA

If there is no marker input terminal on a recorder, the EVENT MARKER signal may be connected in parallel with the recorder input signal to create an event marker in some cases.

## 2.7 Installing the standard test tube rack

---

The standard test tube rack supplied with the WFC III can accommodate test tubes from 12 to 18.1 mm in diameter and 105 to 180 mm in length. The rack cover can be removed from the rack for cleaning.

Other collection vessels may be used and other rack options are available. For additional information, see [“Optional racks” on page 20](#).

### 2.7.1 Test tube supports

The standard tube rack features removable tube supports which hold test tubes that are 12 to 15 mm in diameter upright and centered. Test tubes greater than 15 mm in diameter can be used without the tube supports. When used frequently with larger tubes, the tube supports may become permanently expanded and the smaller test tubes may no longer fit snugly. To avoid this problem, it is recommended that the tube supports be removed when using larger tubes or that an additional rack be purchased if a variety of sizes will be used.

When the rack is cold, the test tube supports will be rigid. To avoid damage to the test tubes or supports, insert and remove tubes with extra care.

**Tip:** Avoid using excessive force against the test tube holders as this may cause distortion, especially when the rack cover is removed.



**Warning:** Do not put your fingers into the holes of the test tube support. Pulling your fingers out quickly can cause injury.

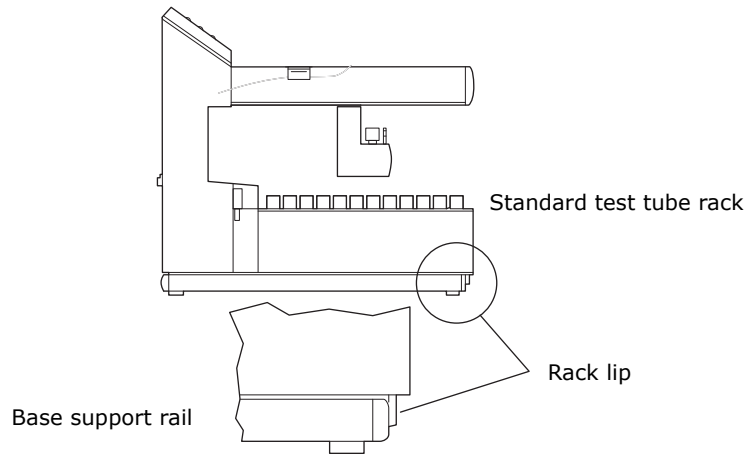
#### To install the standard test tube rack:

1. Hold the rack with tube 1 farthest away from you on the left side.
2. Align the rack with the base support rails.



3. Slide the rack on the base support rails until the front tab of the rack rests firmly against the end of the rails.

**Figure 2-15: Installing the standard test tube rack**



4. Press firmly to ensure the rack is seated fully.
5. Verify that the test tubes are perfectly vertical.

## 2.8 Installing optional racks

---

There are several optional racks available for the WFC III, including:

- Prep funnel rack
- 17-mm vial rack
- 28-mm vial rack
- Micro plate rack
- Eppendorf tube rack
- Carousel rack
- Multi-purpose rack

### 2.8.1 Installing the prep funnel and funnel rack

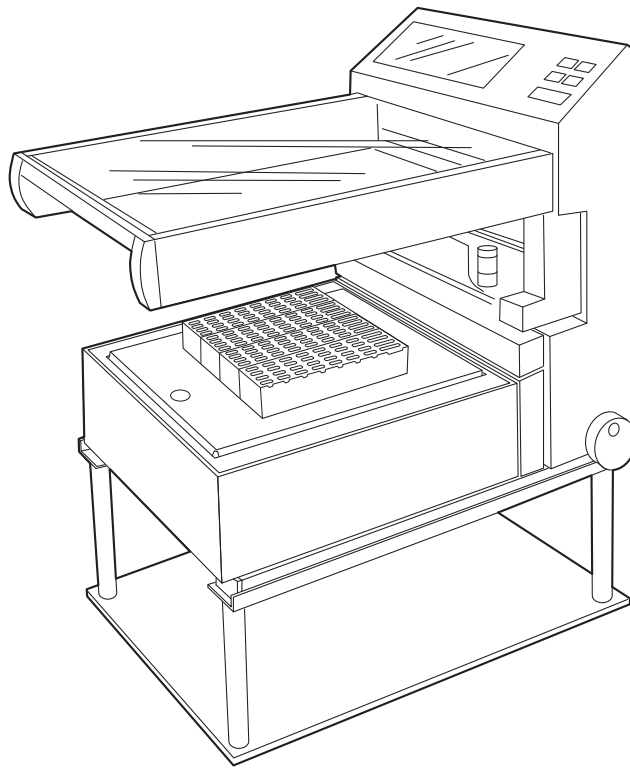
The optional prep funnel rack replaces the standard tube rack and is used to collect samples in up to 128 large bottles. The prep funnel rack must be used in combination with the optional funnel rack and the optional tabletop rack.

- ! **Notice:** The 90-funnel rack used with the WFC II is compatible with the WFC III. However, the WFC III prep funnel collection program does not accommodate the 90-funnel rack used with the WFC II. If you use the 90-funnel rack, you must program the collection pattern using the bottle position function. See the [table titled "Programming bottle positions" on page 56](#), for information about programming the collection pattern.

**To install the prep funnel and funnel rack:**

1. Move the WFC III onto the tabletop rack or other rack that allows you to route tubing to large bottles for collection.

**Figure 2-16: WFC III on tabletop rack**



2. Install the prep funnel rack on the WFC III.
3. Place the collection bottles underneath the tabletop rack.
4. Install one, two, three, or four prep funnels as necessary on the funnel rack beginning on the left side.

**Tip:** If you use fewer than four prep funnels, they must be loaded sequentially on the rack. See the [figure "Dropper assembly movement – prep funnel rack" on page 61](#) for the correct funnel arrangement.

5. Connect a drain tube to the drain outlet and lead the tube to a waste vessel.
6. Connect the sampling tube from a chromatography system to the dropper assembly of the WFC III.

7. Use the height adjustment knob to adjust the carriage to a height appropriate for the rack.
8. Use the RACK SELECTION mode to set the unit for the rack. See ["Selecting the rack" on page 53](#), for more information.

## 2.8.2 Installing the 28-mm vial rack

The optional 28-mm vial rack replaces the standard tube rack. It allows collection of samples into 28-mm OD scintillation vials.

### To install the 28-mm vial rack:

1. Install vials into the vial rack.
2. Install the vial rack on the WFC III.
3. Use the height adjustment knob to adjust the carriage to a height appropriate for the rack.
4. Use the RACK SELECTION mode to set the unit for the rack. See ["Selecting the rack" on page 53](#), for more information.

## 2.8.3 Installing the micro plate rack

The optional micro plate rack replaces the standard tube rack. It allows collection of samples into up to four 96-well micro titer plates. The micro plate rack should be used in combination with the optional extension nozzle (part number 725000151).

**Tip:** You can use standard or deep-well micro titer plates on the micro plate rack, but you cannot mix plate types. Use all standard or all deep-well plates on the rack.

**!** **Notice:** The two-plate micro plate rack used with the WFC II is compatible with the WFC III. However, the WFC III micro plate collection program does not accommodate the two-plate rack used with the WFC II. If you use the two-plate rack, you must program the collection pattern using the bottle position function. See ["Programming bottle positions" on page 56](#), for information about programming the collection pattern.

### To install the extension nozzle:

1. Loosen the tubing collar on the dropper assembly and remove the sample tubing.
2. Insert the metal end of the extension nozzle until it protrudes from the bottom of the mounting plate.
3. Tighten the tubing collar so that the extension nozzle is held firmly in place. Be sure to hand tighten only. Overtightening can strip the threads or otherwise stress the connections.
4. Pass the other end of the tube through the slot in the left side of the dust cover and connect it to your chromatography system.

Once you have installed the extension nozzle, you can install the micro plate rack.

**!** **Notice:** When using Drop Collection Mode with an extension nozzle, the extension nozzle may be too long and could possibly extend below the range of the sensor. If this occurs, the drops from these nozzles may fall below the sensor's ability to read them and none of the drops will get counted. This only occurs with stand-alone units in this mode. If you are controlling the WFC III using software, you will not experience this issue.

#### **To install the micro plate rack:**

1. Install up to four 96-well micro titer plates on the micro plate rack. If you use fewer than four plates, the plates must be loaded sequentially on the rack. See the [figure "Dropper assembly movement – micro plate rack" on page 60](#) for the correct plate arrangement.

**!** **Notice:** Pay careful attention to the arrangement of the plates on the rack. The plates should be arranged with the first well (marked A, 1 on the plate) positioned as indicated by the black corners in the [figure "Dropper assembly movement – micro plate rack" on page 60](#).

2. Install the micro plate rack on the WFC III.
3. Use the height adjustment knob to adjust the carriage to a height appropriate for the rack.
4. Use the RACK SELECTION mode to set the unit for the rack and adjust the position of the dropper assembly over the rack. See ["Selecting the rack" on page 53](#), for more information.

### **2.8.4 Installing the carousel rack**

The optional carousel rack replaces the standard tube rack. It allows for collection of samples into 2-ml vials installed in Waters Alliance carousels.

#### **To install the carousel rack:**

1. Install each carousel on the carousel rack.
2. Install the carousel rack on the WFC III.
3. Use the height adjustment knob to adjust the carriage to a height appropriate for the rack.
4. Use the RACK SELECTION mode to set the unit for the rack and adjust the position of the dropper assembly over the rack. See ["Selecting the rack" on page 53](#), for more information.

### **2.8.5 Installing the 17-mm vial rack**

The optional 17-mm vial rack replaces the standard tube rack. It allows collection of samples into 17-mm OD scintillation vials.

#### **To install the 17-mm vial rack:**

1. Install vials into the vial rack.

2. Install the vial rack on the WFC III.
3. Use the height adjustment knob to adjust the carriage to a height appropriate for the rack.
4. To set the unit for the rack use the RACK SELECTION mode, then select STANDARD. See ["Selecting the rack" on page 53](#), for more information.

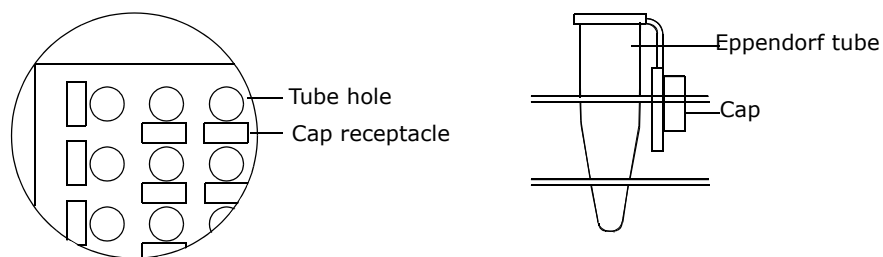
## 2.8.6 Installing the Eppendorf tube rack

The optional Eppendorf tube rack replaces the standard tube rack. It allows collection of samples into Eppendorf tubes.

### To install the Eppendorf tube rack:

1. Insert Eppendorf tubes into the tube holes in the Eppendorf tube rack.
2. Insert the tube caps.

**Figure 2-17: Placement of tubes in the Eppendorf rack**



3. Install the Eppendorf tube rack on the WFC III.
4. Use the height adjustment knob to adjust the carriage to a height appropriate for the rack.
5. To set the unit for the rack use the RACK SELECTION mode, then select STANDARD. See ["Selecting the rack" on page 53](#), for more information.

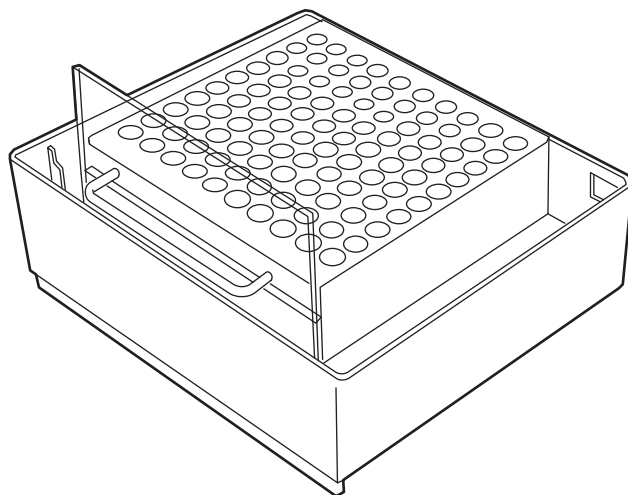
## 2.8.7 Installing the multi-purpose rack

The optional multi-purpose rack replaces the standard test tube rack. It accepts a Waters 2700 Sample Manager rack, up to two Gilson FC 204 Fraction Collector racks, or up to eight GeneVac test tube holders. You can also combine one Gilson FC 204 Fraction Collector rack and up to four GeneVac test tube holders on one multi-purpose rack. After installing the rack, you can use the RACK SELECTION mode and BOTTLE POSITION options to manually set the unit for your rack.

### To install the multi-purpose rack:

1. Install the 2700 Sample Manager rack, Gilson FC 204 Fraction Collector racks, or GeneVac test tube holders on the multi-purpose rack.

**Figure 2-18: Multi-purpose rack with 2700 rack installed**



2. Install the multi-purpose rack on the WFC III.
3. Use the height adjustment knob to adjust the carriage to a height appropriate for the rack.
4. Use the RACK SELECTION mode and BOTTLE POSITION options to set the unit for the rack. See ["Selecting the rack" on page 53](#), for more information.

**Tip:** The multi-purpose rack can also hold up to two Gilson FC 204 Fraction Collector racks or up to eight GeneVac test tube holders.

# 3 Preparation and operation

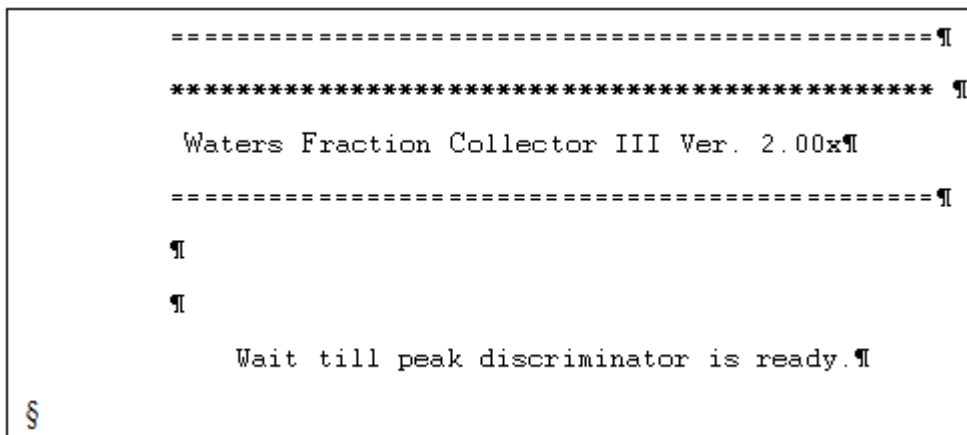
This chapter describes how to set up and use the Waters Fraction Collector III. Read this chapter and perform the preliminary setup procedures before you collect fractions.

## 3.1 Powering on the unit

---

Before you set up the WFC III, power on the unit by moving the power switch (located at the bottom-left side of the rear panel) to the ON position. The LCD is activated and appears similar to the figure below.

**Figure 3–1: Initial LCD display**



To allow for auto-adjustment by the peak discriminator, the LCD display remains as shown for approximately 10 to 15 seconds. Once the auto-adjustment is complete, the unit beeps and the following message appears momentarily:

```
!!! Nozzle moves to ready starting port !!!
```

The dropper assembly then moves to the home position and the MAIN MENU is displayed.

**Figure 3-2: WFC III MAIN MENU**

```
MENU ===== ACTION MODE ===== (STANDARD)¶
=====¶
[TIME] [DROP] [SINGLE] [VOLUME]¶
[PEAK] [WINDOW] [MANUAL] [SAMPLING]¶
¶
----- UTILITY -----¶
[DISPLAY RESULT] [RACK SELECTION]¶
[CONDITIONS] [BOTTLE POSITION]§
```

If the unit experiences a power failure, the message shown appears. To clear the message and move to the next screen, press **ENTER**.

**Figure 3-3: Power failure message**

```
=====¶
*****¶
Waters Fraction Collector III Ver. 2.00x¶
=====¶
¶
Power failed during a run. Press ENTER ¶
§
```

**Tip:** You can use the CONDITIONS mode to select whether or not this message should appear when the power fails. See ["Setting conditions" on page 51](#).

### 3.1.1 Initializing the memory

You can initialize the unit's memory when powering on. Initializing the memory clears all parameter values and reloads the default values.

**To initialize the unit's memory:**

1. Power off the unit by moving the power switch to the **OFF** position.
2. Hold down the **ENTER** and **END** keys and power on the unit.



## 3.2 LCD display and keypad overview

---

You use the LCD display and keypad on the front panel to control the unit.

### 3.2.1 LCD display

Once the unit is powered on and the peak discriminator has auto-adjusted, the MAIN MENU appears on the LCD window. The MAIN MENU shows all mode options except the TEST mode. To make a selection, use the **UP**, **DOWN**, **←**, and **→** keys on the keypad to move the cursor until the name of the desired selection is highlighted, then press **ENTER**.

The brightness of the LCD can be adjusted using the CONDITIONS mode (see [“Setting conditions” on page 51](#)).

#### 3.2.1.1 Changing the LCD display language

By default, the WFC III’s display language is English. The unit is also capable of Japanese display.

##### To change the display language to Japanese:

1. Power off the unit.
2. Hold down the **←** key and power on the unit. The display appears in Japanese.

##### To change the display language to English:

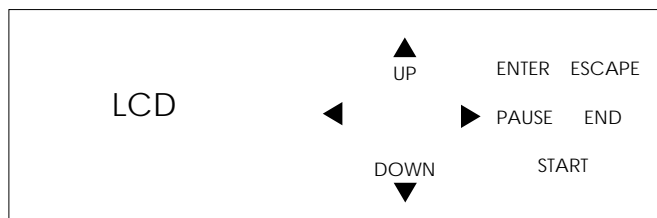
1. Power off the unit.
2. Hold down the **→** key and power on the unit. The display appears in English.

**Tip:** The display language can also be changed using the CONDITIONS mode. See [“Setting conditions” on page 51](#).

### 3.2.2 Keypad functions

In addition to the LCD display, the front panel of the Fraction Collector III has nine pressure-sensitive keys. The [table titled “Keypad functions” on page 50](#) lists the function of each key during general operation. (Other functions are described in the individual mode sections where applicable.)

**Figure 3–4: Front panel keys**



**Table 3–1: Keypad functions**

Key	Main function
UP	Moves the cursor one row up on the LCD. Increases preset numbers one digit at a time.
DOWN	Moves the cursor one row down on the LCD. Decreases preset numbers one digit at a time.
®	Moves the cursor one selection to the right on the LCD.
↶	Moves the cursor one selection to the left on the LCD.
ENTER	Activates the selected menu choice. When entering parameter values, ENTER confirms user input.
ESCAPE	Jumps the cursor to [to MENU] on most screens. When entering parameter values, ESCAPE cancels user input.
PAUSE	Temporarily halts sample collection.
END	Terminates sample collection.
START	Begins sample collection.

To rapidly increase or decrease preset numbers, press **START** while holding down the **UP** or **DOWN** key.

! **Notice:** Remember to use only finger pressure to operate the keys. Do not use sharp or metallic objects to press the keys.

### 3.3 Preliminary setup

---

Before you begin collecting fractions you need to perform some preliminary setup of the WFC III. Although you specify these options before you begin to use the WFC III, you may need to reset some options before each collection run. You use the following Utility modes to prepare the WFC III for fraction collection:

- CONDITIONS
- RACK SELECTION
- BOTTLE POSITION

The DISPLAY RESULT mode, which allows you to review the previous run as a table or a graph displayed on the LCD window, is covered in ["Displaying the results of a run" on page 104](#).

The TEST mode, which allows you to perform diagnostic tests on the WFC III, is covered in ["Running the test mode diagnostics" on page 110](#).

### 3.3.1 Setting conditions

In CONDITIONS mode, various instrument functions common to most modes can be controlled. These functions include input ranges and speeds, display language and contrast, and some types of dropper movement.

The CONDITIONS mode has two menus, CONDITIONS (1) and CONDITIONS (2), which list the CONDITIONS parameters. Current selections are indicated on the menus with an asterisk (\*).

To access the CONDITIONS (1) menu, select **CONDITIONS** on the MAIN MENU and press **ENTER**. The CONDITIONS (1) menu appears.

**Figure 3-5: CONDITIONS (1) menu**

=====CONDITIONS(1)=====	
signal level:\$	[*10 mV] [1V]\$
samp.flow rate:\$	[10.0] ml / min\$
multi.s skip:\$	[*ON] [OFF] at restart\$
3Way Valve:\$	attached\$
\$	drain [*SAMPLE] [DRAIN]\$
\$	\$
[CONDITIONS (2)] [to MENU]\$	

The table below lists the CONDITIONS (1) parameters.

**Table 3-2: CONDITIONS (1) parameters**

Parameter	Description
signal level	Select an input range of 10 mV or 1 V FS (full scale) depending on the peak detection device used.
samp.flow rate	Allows you to input sample flow rate of 0.1 ml to 999.9 ml/min. This is used for calculating time per fraction in VOLUME mode.
multi.s skip	Allows you to choose whether to skip one collection vessel between collection cycles in multi-sample mode.
3 Way Valve	When a 3-way valve is installed, the screen displays ATTACHED. Otherwise, NON ATTACH is displayed. You cannot modify this parameter; it is automatically changed when the 3-way valve is installed or removed.

**Table 3–2: CONDITIONS (1) parameters (continued)**

Parameter	Description
3 Way Valve drain position	If a 3-way valve is installed, choose either SAMPLE or DRAIN to determine how the sample is diverted during draining. If DRAIN is chosen, the dropper assembly moves to a position over the drain and drains through the dropper opening. If SAMPLE is chosen, draining occurs through the NO (normally open) drain port of the 3-way valve while the dropper assembly stays over the collection vessel. This option appears only if the 3-way valve is installed.

**To set the CONDITIONS (1) parameters:**

1. On the MAIN MENU, select **CONDITIONS** and press **ENTER**. The CONDITIONS (1) menu appears.
2. Use the ← and → keys to select the desired signal level and press **ENTER**. The cursor moves to the sample flow rate value.
3. Use the **UP** and **DOWN** keys to enter the desired sample flow rate and press **ENTER**. The cursor moves to the multi sample skip value.
4. Use the ← and → keys to select the desired multi-sample skip option and press **ENTER**. The cursor moves to CONDITIONS (2).
5. Press **ENTER** to display the CONDITIONS (2) menu or select **[to MENU]** and press **ENTER** to return to the MAIN MENU.

To access the CONDITIONS (2) menu, select **CONDITIONS (2)** on the CONDITIONS (1) menu and press **ENTER**. The menu shown below is displayed.

**Figure 3–6: CONDITIONS (2) menu**

=====CONDITIONS (2)=====	
language:\$	[*ENGLISH] [JAPANESE]\$
buzzer:\$	[*ON] [OFF] at nozzle move\$
\$	[*ON] [OFF] at end of run\$
rs232c speed:\$	[12] [24] [*48] x 100 bps\$
pwr on message:\$	[*ON] [OFF] at pw. failed\$
LCD contrast:\$	[64] (0 - 128)\$
[CONDITIONS (1)] [to MENU]\$	

The table titled “CONDITIONS (2) parameters” on page 53 lists the CONDITIONS (2) parameters.

**Table 3–3: CONDITIONS (2) parameters**

<b>Parameter</b>	<b>Description</b>
language	English or Japanese can be selected.
buzzer [*ON] at nozzle move	If this is selected, the unit beeps when the dropper assembly is in motion.
buzzer [*ON] at end of run	If this is selected, the unit beeps three times at the end of a run.
rs232c speed	Selects the speed of installed RS-232C (select 1200, 2400, or 4800 bps). This parameter should be used with the MassLynx software. Select 4800 on the WFC III and within the MassLynx software.
pwr on message	Default [ON] displays the message "Power failed during a run. Press ENTER" if power is interrupted during instrument operation. Select [ <b>OFF</b> ] to suppress this message.
LCD contrast	Allows you to adjust the brightness of the LCD.

**To set the CONDITIONS (2) parameters:**

1. On the CONDITIONS (1) menu, select **CONDITIONS (2)** and press **ENTER**. The CONDITIONS (2) menu appears.
2. Use the ← and → keys to select the desired display language and press **ENTER**. The cursor moves to the buzzer option.
3. Use the ← and → keys to select the desired buzzer option for nozzle moves and press **ENTER**. The cursor moves to the end of run buzzer option.
4. Use the ← and → keys to select the desired buzzer option for the end of a run and press **ENTER**. The cursor moves to the rs232c speed option.
5. Press **ENTER**. The cursor moves to the power on message option.
6. Use the ← and → keys to select the desired power on message option and press **ENTER**. The cursor moves to the LCD contrast value.
7. Use the **UP** and **DOWN** keys to enter the desired LCD contrast value and press **ENTER**. The cursor moves to CONDITIONS (1).
8. Press **ENTER** to display the CONDITIONS (1) menu or select [**to MENU**] and press **ENTER** to return to the MAIN MENU.

### **3.3.2 Selecting the rack**

In addition to the standard rack, the WFC III allows you to use alternate arrangements of bottles or other collection vessels with the top removed from the standard rack, or you can purchase specialized racks for particular types of collection vessels. The RACK SELECTION mode sets the WFC III for the type of rack installed or bottle arrangement used.

! **Notice:** The MICRO TP4, formerly MICRO PLT, rack selection is designed for use only with the four-plate micro plate rack. It does not accommodate the two-plate rack used with the WFC II. If you use the two-plate rack, you must program the collection pattern using the bottle position function. See ["Programming bottle positions" on page 56](#), for information about programming the collection pattern.

! **Notice:** The FUNNEL128, formerly FUNNEL RC, rack selection is designed for use only with the 128-funnel rack. It does not accommodate the 90-funnel rack used with the WFC II. If you use the 90-funnel rack, you must program the collection pattern using the bottle position function. See ["Programming bottle positions" on page 56](#), for information about programming the collection pattern.

The figure below shows the RACK SELECTION menu.

**Figure 3-7: RACK SELECTION menu**

-----RACK SELECTION-----		
RACK :\$	[*STANDARD]\$	[MICRO TP4]\$
\$	[VIAL 28 mm]\$	[FUNNEL128]\$
\$	[CAROUSEL]\$	\$
\$	[BOTTLE-1-]\$	[BOTTLE-2-]\$
\$	[BOTTLE-3-]\$	\$
[to MENU]\$	[RACK ADJUSTMENT]\$	

**To set the WFC III for the rack installed:**

1. In the MAIN MENU, move the cursor to **RACK SELECTION** and press **ENTER**. The RACK SELECTION menu appears.
2. Use the **UP**, **DOWN**, **←**, and **→** keys to select the desired rack, then press **ENTER**.

**Tip:** If you are using the optional 17-mm vial rack or Eppendorf tube rack, select the standard rack.

**Tip:** BOTTLE-1, BOTTLE-2, or BOTTLE-3 can be selected only after the bottle parameters have been entered using the BOTTLE POSITION mode. See ["Programming bottle positions" on page 56](#), for instructions on how to enter bottle parameters.

3. Perform one of these steps to complete the rack selection:
  - If you selected the micro plate or carousel rack, see ["Micro plate and carousel racks" on page 55](#) below.
  - If you selected the multi-purpose rack, see ["Programming bottle positions" on page 56](#).

- If you selected a rack other than the micro plate, carousel, or multi-purpose rack, move the cursor to **[to MENU]** and press **ENTER** to return to the MAIN MENU.

### 3.3.2.1 Micro plate and carousel racks

When MICRO TP4 or CAROUSEL is selected on the RACK SELECTION menu, the RACK ADJUSTMENT menu appears. The RACK ADJUSTMENT menu allows you to adjust the position of the dropper assembly over the micro plate or carousel rack.

**Figure 3–8: RACK ADJUSTMENT menu**

=====RACK	ADJUSTMENT=====
NOZZLE MOVES :\$	[No. 1] UP or DOWN = moves\$
\$	\$
ADJUSTMENT :\$	[*] x+0.0 mm Y + 0.0 mm\$
\$	\$
\$	\$
[to MENU]\$	\$

#### To adjust the position of the dropper assembly over the micro plate or carousel rack:

1. Install the micro plate or carousel rack.
2. Follow the previous instructions for selecting the micro plate or carousel rack in the RACK SELECTION menu.
3. On the RACK SELECTION menu, move the cursor to **RACK ADJUSTMENT** and press **ENTER**. The RACK ADJUSTMENT menu appears and the dropper assembly moves to the default first position.
4. Move the cursor to **[\*]** and use the **UP**, **DOWN**, **←**, and **→** keys to align the dropper assembly over the first position on the rack. You can move the dropper assembly by as little as 0.1 mm at a time, up to 5.0 mm in each direction.
5. When the dropper assembly is in the desired position, press **ENTER**.
6. Move the cursor to **[No. 1]** and press **UP** to move the dropper assembly to the next position (position 89 if you selected the micro plate rack, position 7 if you selected the carousel rack). Align the dropper assembly over the collection vessel.
7. Repeat steps 4 through 6 to align the dropper assembly over the remaining positions (97, 185, 193, 281, 289, and 377 for the micro plate rack and 13, 19, 25, 31, 37, and 43 for the carousel rack).
8. When you have adjusted the dropper assembly at all positions, the dropper assembly moves to the first position. Select **[to MENU]** and press **ENTER** to return to the MAIN MENU.

### 3.3.3 Programming bottle positions

The WFC III allows you to collect samples in a regular array or arbitrary setup of bottles or other nonstandard collection vessels. In order for the dropper assembly to move to the correct positions, the bottle arrangement must be programmed in BOTTLE POSITION mode. Up to three custom arrangements can be entered and stored in BOTTLE POSITION mode.

Before you can select a custom arrangement on the RACK SELECTION menu, you must use the BOTTLE POSITION menu to create the arrangement. The figure below shows the BOTTLE POSITION menu.

**Figure 3-9: BOTTLE POSITION menu**

=====BOTTLE POSITION=====		
BOTTLE-1-: \$	[*regular]\$	[arbitrary]\$
\$	10 x12 (120)\$	8 x 5 (40)\$
BOTTLE-2-: \$	[*regular]\$	[arbitrary]\$
\$	\$	(100)\$
BOTTLE-3-: \$	[*regular]\$	[arbitrary]\$
\$	===== \$	\$
[to MENU]\$	\$	\$

Use the regular option (see the [figure "BOTTLE POSITION screen \(regular option\)" on page 57](#)) when you have one or two evenly spaced arrays of bottles. Use the arbitrary option (see the [figure "BOTTLE POSITION screen \(arbitrary option\)" on page 58](#)) when the bottles vary in size or are offset.

#### 3.3.3.1 Regular option

The regular option allows you to program bottle arrangements with evenly spaced arrays of bottles. Each arrangement can contain one or two arrays.

##### To program a bottle arrangement using the regular option:

1. On the MAIN MENU, select **BOTTLE POSITION** and press **ENTER**. The BOTTLE POSITION menu appears.
2. On the BOTTLE POSITION menu, select **BOTTLE 1 [regular]** and press **ENTER**. The BOTTLE POSITION screen (Regular) appears and the dropper assembly moves to the home position.



**Figure 3–10: BOTTLE POSITION screen (regular option)**

====BOTTLE POSITION=====BOTTLE-1-(regular)=====	\$
regular:\$	\$
X-positions:\$	<-----[10]----->\$
origin:\$	[*] X (123.4) Y (123.4)\$
Y-positions:\$	[10]\$
\$	\$
opposite:\$	X (123.4) Y (123.4) [*]\$
[regular 2] [BOTTLE POSITION]\$	

3. Move the cursor to **X-positions [ ]**.
4. Use the **UP** and **DOWN** keys to enter the **number of bottles** in the X-direction (left to right).
5. Move the cursor to **Y-positions [ ]**.
6. Use the **UP** and **DOWN** keys to enter the **number of bottles** in the Y-direction (front to back).
7. Move the cursor to **origin [\*]**.
8. Use the **UP**, **DOWN**, **←**, and **→** keys to center the dropper assembly over the first bottle in the array and press **ENTER**. (The first bottle is the one closest to the home position.)
9. Move the cursor to **opposite [\*]**.
10. Use the **UP**, **DOWN**, **←**, and **→** keys to center the dropper assembly over the bottle in the opposite corner of the array (farthest from the first bottle) and press **ENTER**. The bottle arrangement will be stored for this parameter set.
11. To program a second array of bottles on the same rack, select **[regular 2]** and press **ENTER**. Then follow steps 3 through 10.

### 3.3.3.2 Arbitrary option

The arbitrary option allows you to program arrangements of bottles that vary in size or are positioned randomly. The arrangement can contain up to 110 bottle positions.

#### To program a bottle arrangement using the arbitrary option:

1. On the MAIN MENU, select **BOTTLE POSITION** and press **ENTER**. The BOTTLE POSITION menu appears.
2. On the BOTTLE POSITION menu, select **BOTTLE 1 [arbitrary]** and press **ENTER**. The BOTTLE POSITION screen (Arbitrary) appears and the dropper assembly moves to the home position.

**Figure 3–11: BOTTLE POSITION screen (arbitrary option)**

====BOTTLE POSITION=====BOTTLE-1-(regular)=====	
regular:\$	\$
X-positions:\$	<-----[10]----->\$
origin:\$	[*] X (123.4) Y (123.4)\$
Y-positions:\$	[10]\$
\$	\$
opposite:\$	X (123.4) Y (123.4) [*]\$
[regular 2] [BOTTLE POSITION]\$	

3. Move the cursor to the position **[\*]** for the first bottle number.
4. Use the **UP**, **DOWN**, **←**, and **→** keys to center the dropper assembly over the bottle. The bottle coordinates are displayed in mm from the home position.
5. Press **ENTER** to confirm the bottle position (or **ESCAPE** to return to the home position).
6. Select position **[\*]** for the next bottle. The coordinates for the previous bottle are displayed.
7. Center the dropper assembly over the bottle and press **ENTER**. Repeat steps 4 and 5 until all bottle positions are entered.
8. After you have entered the last bottle position, select position **[\*]** for the next bottle and press **END**. All bottle coordinates after your last entry will be cleared.
9. Press **ESCAPE** to return to the BOTTLE POSITION menu.

**Tip:** You cannot set the dropper position to X = 0.0, Y = 0.0 mm. The initial position must be at least X = 0.1, Y = 0.0 or X = 0.0, Y = 0.1.

### 3.3.4 Dropper assembly movement

The WFC III delivers fluid to collection vessels through the dropper assembly. The dropper assembly is attached to the carriage, which moves back and forth along the carriage rails. The dropper assembly also moves left and right along the carriage. These movements allow the dropper assembly to be positioned directly over the collection vessels in a preset pattern according to the rack selected in RACK SELECTION mode.

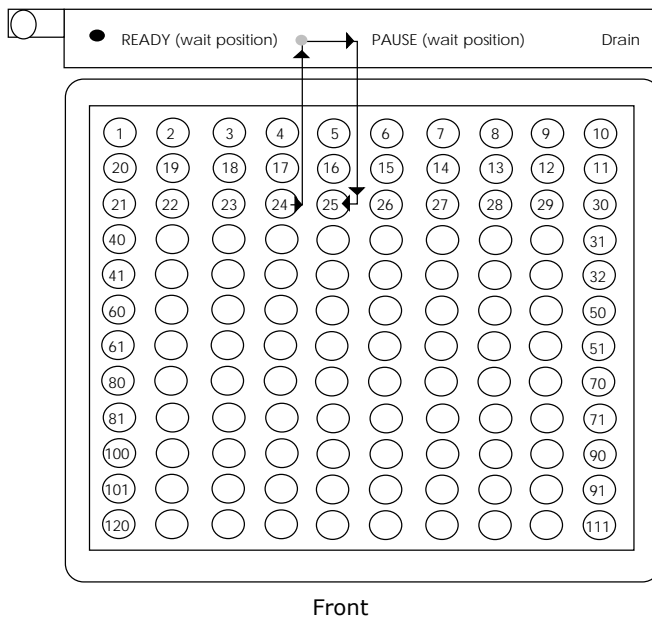
When the unit is powered on, the dropper assembly moves to the ready position (located in the back-left corner, over the drain tray). The dropper assembly remains in the ready position until the run begins and returns to the ready position when the run is complete.

When using the standard test tube rack and 28-mm vial rack in the PAUSE mode, the dropper assembly moves between collection vessels to the drain position. With other racks, the dropper assembly moves over the collection vessels to the drain position.

The figure “Dropper assembly movement – standard test tube rack, 17-mm vial rack, and Eppendorf tube rack” on page 59 shows the collection pattern for the standard test tube rack and the optional 17-mm vial rack and Eppendorf tube rack. Collection proceeds sequentially up to the preset number of test tubes (fractionating No.), to a maximum of 120 tubes. If the fractionating No. is set to 0, the WFC III repeats the cycle from 1 to 120 until an end signal is received.

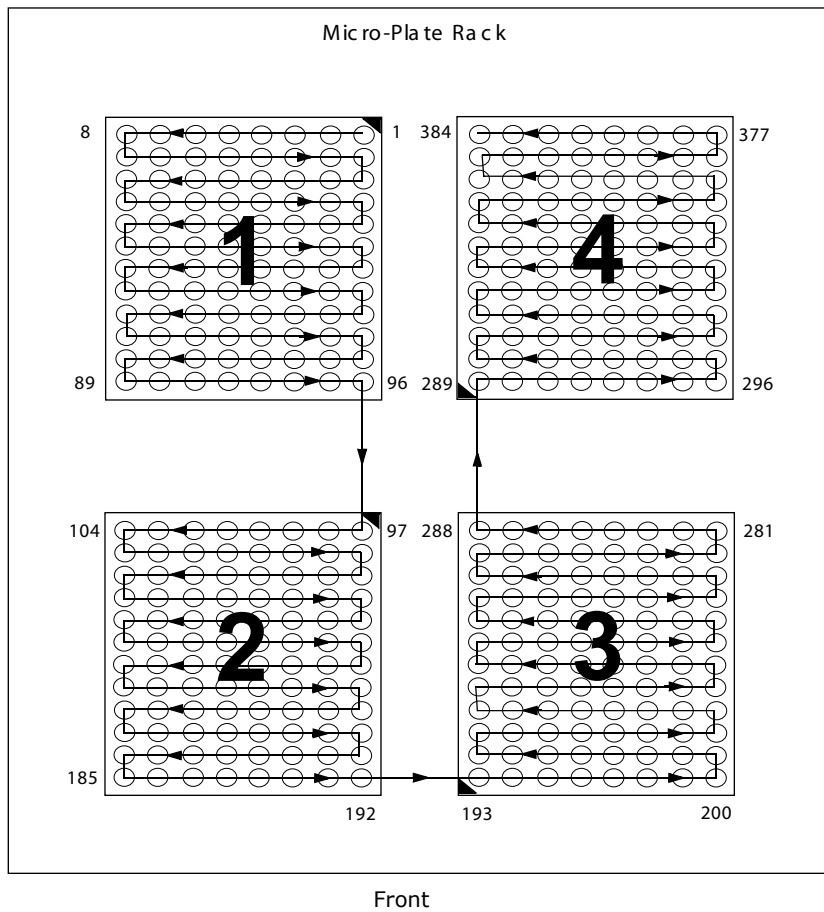
**Tip:** The figures shown in this section are representative pictures intended to document the dropper movement patterns.

**Figure 3–12: Dropper assembly movement – standard test tube rack, 17-mm vial rack, and Eppendorf tube rack**



The figure below shows the collection pattern for the optional micro plate rack.

**Figure 3–13: Dropper assembly movement – micro plate rack**

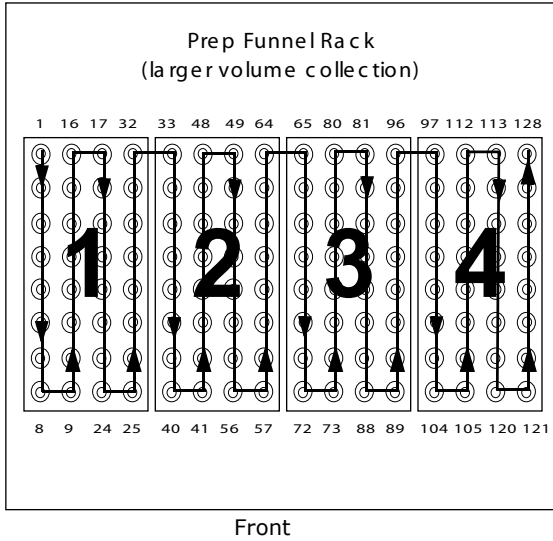


If desired, you can load fewer than four micro plates on the micro plate rack. However, the plates must be loaded sequentially on the rack. For instance, to use only three plates, you must load the plates in positions 1, 2, and 3. If you are only using two plates, install the plates in positions 1 and 2.

**!** **Notice:** When using the micro plate rack, pay careful attention to the arrangement of the plates on the rack. Arrange the plates with the first well (marked A, 1 on the plate) positioned as indicated by the black corners in the [figure "Dropper assembly movement – micro plate rack" on page 60](#).

The figure below shows the collection pattern for the optional prep funnel rack.

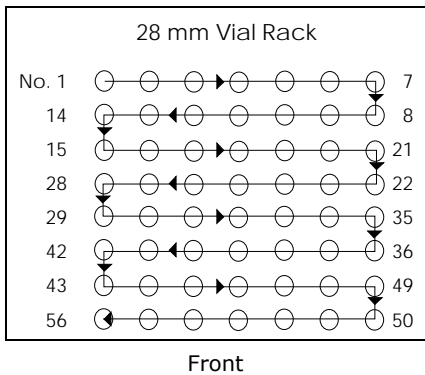
**Figure 3–14: Dropper assembly movement – prep funnel rack**



If desired, you can load fewer than four prep funnels on the prep funnel rack. However, the funnels must be loaded in order on the rack. For instance, to use only three funnels, you must load the funnels in positions 1, 2, and 3.

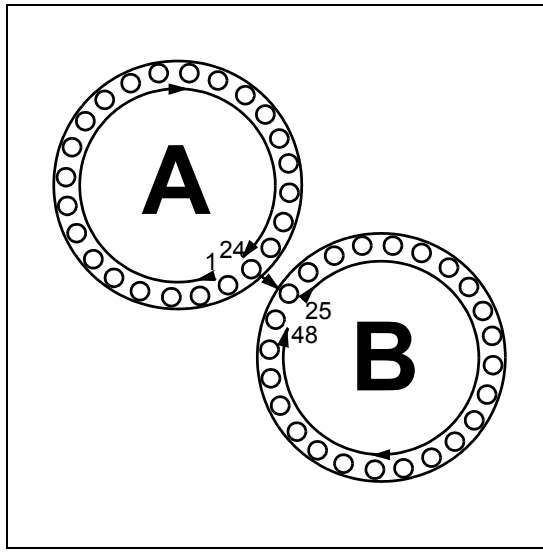
The figure below shows the collection pattern for the optional 28-mm vial rack.

**Figure 3–15: Dropper assembly movement – 28-mm vial rack**



The figure below shows the collection pattern for the optional carousel rack.

**Figure 3-16: Dropper assembly movement – carousel rack**



Front

# 4 Collecting Fractions Using Simple Modes

This chapter describes how to use the Waters Fraction Collector III's simple modes to collect fractions. Simple modes, which are described in "Collecting using time" on page 63, to "Collecting using volume" on page 75, include:

- TIME
- DROP
- SIGNAL
- VOLUME

## 4.1 Collecting using time

---

TIME mode allows you to collect fractions by setting a collection time interval. During a TIME mode collection run, the sample flows into each collection vessel for the specified collection time. The volume of the collected fractions will be constant provided the flow rate is constant.

### 4.1.1 Setting TIME parameters

You program a TIME mode collection run by assigning values to the input parameters shown in the table below. You can enter and save up to three sets of parameters.

**Table 4-1: TIME mode input parameters**

Parameter	Default	Range	Description
parameter No.	1	1 to 3	Identifies the set of input parameters.
time.fraction	[0 min, 10 sec]/tube	0 to [99 min, 59 sec]/tube	Time allotted for the collection per collection vessel.

**Table 4–1: TIME mode input parameters (continued)**

<b>Parameter</b>	<b>Default</b>	<b>Range</b>	<b>Description</b>
fractionating No.	1	1 to 384, depending on rack selected	Number of samples you want to collect in the run. Entering 0 for the fractionating number programs the unit to begin collecting again at position 1 after filling all collection vessels in the rack. The unit repeats the cycle until an end signal is given.
wait.time	0	0 to 99 min, 59 sec	Time interval between the start signal and the beginning of sample collection in the first collection vessel.
multi sample	SINGLE	SINGLE or MULTI	Allows collection of multiple runs with the same parameters in one rack, such as when using an autosampler.

**To enter the TIME mode input parameters:**

1. On the MAIN MENU, select **TIME** and press **ENTER**. The TIME MODE menu appears.
2. Move the cursor to the [ ] to the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The current values for the selected parameter set are displayed and the cursor moves to the time.fraction value.
4. Use the **UP** and **DOWN** keys to enter the desired time.fraction value and press **ENTER**. The cursor moves to the fractionating No. value.
5. Use the **UP** and **DOWN** keys to enter the desired fractionating No. value and press **ENTER**. The cursor moves to the wait.time value.
6. Use the **UP** and **DOWN** keys to enter the desired wait.time value and press **ENTER**. The cursor moves to the multi sample option.



7. Use the ← and → keys to select the desired value and press **ENTER**. The cursor moves to the top of the TIME MODE menu and the status changes to READY.

Once you have set the input parameters, you can start the collection run, enter another set of parameters, or return to the MAIN MENU.

## 4.1.2 Running the program

Once you have set the input parameters, you collect samples by running the program.

### To run a TIME mode program:

1. Set the TIME mode input parameters as desired.
2. On the TIME MODE menu, verify that the status is READY. If SET appears instead of READY, press **ESCAPE**. The status changes to READY.
3. Press **START** or send the unit an external start command.

Once the program begins, the following occurs:

- When the program begins its cycle, READY changes to RUN.
- The cursor blinks in the upper-left corner of the LCD.
- Wait.time counts down to 0, then changes to elapsed.time as the program progresses.
- Time.fraction counts down. When the counter reaches 0, the dropper assembly moves to the next collection vessel.
- With each consecutive sampling, the fractionate ratio changes accordingly.
- Sample collection finishes when the final fraction is collected, you press END, or when you input an external end signal. When the program ends, the TIME MODE menu reappears.

After the run is complete the WFC III can display a table containing the run results. See ["Displaying the results of a run" on page 104](#), to learn how to display run results.

### 4.1.2.1 Multiple sample runs

When you run a program in multi-sample mode, the menu will be displayed upon completion of each sampling run.

**Figure 4-1: Multi-sample run display**

```
----- TIME MODE ----- (NEXT) STANDARD¶
wait.time           : 0 min 0 sec¶
¶
time.fraction       : 12 min 12 sec¶
fractionate No.     : 120/120    multi sample: 2¶
----- ¶
START = restart (for multi sample)¶
$
```

To stop sample collection and end the program, press **END**, input **EXT.END**, or use an external command.

To continue the program with the next sample run, press **START**, input **EXT. START**, or use an external command. The program then counts down the preset wait time and begins sampling again from the next collection vessel. If the skip option has been selected, the program bypasses one collection vessel, then begins collection.

**Tip:** The skip option is available only when the multi-sample mode is selected.

#### 4.1.2.2 Pausing the program

You can temporarily stop a running TIME mode program. To temporarily stop the program, press **PAUSE**. While the program is paused:

- Elapsed time continues to run.
- The dropper assembly moves to the drain position. If a 3-way valve is installed you can choose to have the dropper assembly pause over the current collection vessel instead of the drain. You select this option in CONDITIONS mode.
- The time for each sampling (time.fraction) and the number of collection vessels (fractionating No.) can be changed.

To restart a paused program, press **START** or send an external start command. The unit resumes collection with the next collection vessel in the preset configuration.

To resume a paused program in multi-sample mode, press **START** to resume the same run (set of fractions) or press **END**, then press **START** to start a new run (set of fractions).

### 4.1.3 Sample TIME mode program

You can familiarize yourself with the TIME mode by entering and running the sample program provided below. The table below shows the input parameter values for the sample program.

**Figure 4-2: TIME mode sample program parameters**

Parameter	Value
time.fraction	10 sec
fractionating No.	5
wait.time	20 sec
multi-sample	MULTI
skip	ON

When you run the sample program, the dropper assembly holds in the ready position over the drain for 20 seconds before it moves to collect 10-second fractions in collection vessels 1 through 5. The dropper assembly then returns to the ready position. A new sample is injected, the start signal is relayed, and the dropper assembly again holds for 20 seconds in the ready position. The dropper assembly then skips collection vessel 6 before collecting five more 10-second fractions in collection vessels 7 through 11.

After the wait time has elapsed and the program begins, the display changes to the example shown in the [figure "Sample program run display" on page 67](#). Elapsed time, time.fraction, and fractionate values change as the program progresses.

**Figure 4-3: Sample program run display**

```
----- TIME  MODE ----- ( RUN ) STANDARD¶
wait.time           : 0 min 5 sec¶
¶
time.fraction       : 0 min 5 sec¶
fractionate         : 1/5      multi sample 1¶
----- ¶
UP = advance to the next point$
```

#### To set up the sample program:

1. On the MAIN MENU, select **TIME** and press **ENTER**. The TIME MODE menu appears on the LCD.
2. Move the cursor to the [ ] at the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The cursor moves to time.fraction.

4. Use the **UP** and **DOWN** keys to enter **0 min 10 sec** as the time.fraction value and press **ENTER**. The cursor moves to fractionating No.
5. Use the **UP** and **DOWN** keys to enter **5** as the fractionating No. value and press **ENTER**. The cursor moves to wait.time.
6. Use the **UP** and **DOWN** keys to enter **0 min 20 sec** as the wait.time value. Press **ENTER** to confirm input and move the cursor to multi sample.
7. Use the → key to select **multi** and press **ENTER**. The cursor moves to the top of the menu.
8. Move the cursor to **[to MENU]** and press **ENTER**. The MAIN MENU reappears.
9. Move the cursor to **CONDITIONS** and press **ENTER**. The CONDITIONS menu appears.
10. Move the cursor to **multi.s skip [ON]** and press **ENTER**.
11. Move the cursor to **[to MENU]** and press **ENTER**. The MAIN MENU reappears.

The sample program is now ready to run. Follow the instructions in ["Running the program" on page 65](#), to run the program.

## 4.2 Collecting using drop count

---

In DROP mode, a sensor light, located in the carriage arm, detects the passage of individual drops of effluent. The unit moves to collect the next fraction when the drop count reaches a preset number determined by the user.

### 4.2.1 Setting DROP Parameters

You program a DROP mode collection run by assigning values to the input parameters shown in the table below. You can enter and save up to three sets of parameters.

**Table 4–2: DROP mode input parameters**

Parameter	Default	Range	Description
parameter No.	1	1 to 3	Identifies the set of input parameters.
drop.fraction	10 drops/tube	0 to 9999 drops/tube	Number of drops collected per collection vessel.
fractionating No.	1	1 to 384, depending on rack selected	Number of samples you want to collect in the run. Entering 0 for the fractionating number programs the unit to begin collecting again at position 1 after filling all collection vessels in the rack. The unit repeats the cycle until an end signal is given.

**Table 4–2: DROP mode input parameters (continued)**

Parameter	Default	Range	Description
wait.time	0	0 to 99 min, 59 sec	Time interval between the start signal and the beginning of sample collection in the first collection vessel.
multi sample	SINGLE	SINGLE or MULTI	Allows collection of multiple runs with the same parameters in one rack, such as when using an autosampler.

**To enter the DROP mode input parameters:**

1. On the MAIN MENU, select **DROP** and press **ENTER**. The DROP MODE menu appears.
2. Move the cursor to the [ ] to the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The current values for the selected parameter set are displayed and the cursor moves to the drop.fraction value.
4. Use the **UP** and **DOWN** keys to enter the desired drop.fraction value and press **ENTER**. The cursor moves to the fractionating No. value.
5. Use the **UP** and **DOWN** keys to enter the desired fractionating No. value and press **ENTER**. The cursor moves to the wait.time value.
6. Use the **UP** and **DOWN** keys to enter the desired wait.time value and press **ENTER**. The cursor moves to the multi sample option.
7. Use the ← and → keys to select the desired value and press **ENTER**. The cursor moves to the top of the DROP MODE menu and the status changes to READY.

Once you have set the input parameters, you can start the collection run, enter another set of parameters, or return to the MAIN MENU.

## 4.2.2 Running the program

Once you have set the input parameters, you collect samples by running the program.

**To run a DROP mode program:**

1. Set the DROP mode input parameters as desired.
2. On the DROP MODE menu, verify that the status is READY. If SET appears instead of READY, press **ESCAPE**. The status changes to READY.
3. Press **START** or send the unit an external start command.

Once the program begins, the following occurs:

- When the program begins its cycle, READY changes to RUN.
- The cursor blinks in the upper-left corner of the LCD.

- Wait.time counts down to 0, then changes to elapsed.time as the program progresses.
- Drop.fraction counts down. When the counter reaches 0, the dropper assembly moves to the next collection vessel.
- With each consecutive sampling, the fractionate ratio changes accordingly.
- Sample collection finishes when the final fraction is collected, you press END, or when you input an external end signal. When the program ends, the DROP MODE menu reappears.

After the run is complete the WFC III can display a table containing the run results. See ["Displaying the results of a run" on page 104](#), to learn how to display run results.

#### 4.2.2.1 Multiple sample runs

When you run a program in multi-sample mode, a menu similar to the one shown below is displayed upon completion of each sampling run.

To stop sample collection and end the program, press **END**, input **EXT.END**, or use an external command.

To continue the program with the next sample run, press **START**, input **EXT. START**, or use an external command. The program then counts down the preset wait time and begins sampling again from the next collection vessel. If the skip option has been selected, the program bypasses one collection vessel, then begins collection.

**Tip:** The skip option is available only when the multi-sample mode is selected.

#### 4.2.2.2 Pausing the program

You can temporarily stop a running DROP mode program. To temporarily stop the program, press **PAUSE**. While the program is paused:

- Elapsed time continues to run.
- The dropper assembly moves to the drain position. If a 3-way valve is installed you can choose to have the dropper assembly pause over the current collection vessel instead of the drain. You select this option in CONDITIONS mode.
- The drop count for each sampling (drop.fraction) and the number of collection vessels (fractionating No.) can be changed.

To restart a paused program, press **START** or send an external start command. The unit resumes collection with the next collection vessel in the preset configuration.

To resume a paused program in multi-sample mode, press **START** to resume the same run (set of fractions) or press **END**, then press **START** to start a new run (set of fractions).

#### 4.2.3 Sample DROP mode program

You can familiarize yourself with the DROP mode by entering and running the sample program provided below. The table below shows the input parameter values for the sample program.

**Table 4–3: DROP mode sample program parameters**

Parameter	Value
drop.fraction	50 drops/tube
fractionating No.	4
wait.time	15 sec
multi sample	SINGLE

When you run the sample program, the dropper assembly holds in the ready position over the drain for 15 seconds before it moves to collect 50-drop fractions in collection vessels 1 through 4. The dropper assembly then returns to the ready position and the run ends.

After the wait time has elapsed and the program begins, a screen similar to the example shown in the [figure "Sample program run display" on page 67](#) is displayed. Elapsed time, drop.fraction, and fractionate values change as the program progresses.

**To set up the sample program:**

1. On the MAIN MENU, select **DROP** and press **ENTER**. The DROP MODE menu appears on the LCD.
2. Move the cursor to the [ ] at the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The cursor moves to drop.fraction.
4. Use the **UP** and **DOWN** keys to enter **50** as the drop.fraction value and press **ENTER**. The cursor moves to fractionating No.
5. Use the **UP** and **DOWN** keys to enter **4** as the fractionating No. value and press **ENTER**. The cursor moves to wait.time.
6. Use the **UP** and **DOWN** keys to enter **0 min 15 sec** as the wait.time value. Press **ENTER** to confirm input and move the cursor to multi sample.
7. Use the → key to select **SINGLE** and press **ENTER**. The cursor moves to the top of the DROP MODE menu.
8. Move the cursor to **[to MENU]** and press **ENTER**. The MAIN MENU reappears.

The sample program is now ready to run. Follow the instructions in ["Running the program" on page 69](#), to run the program.

## 4.3 Collecting using signal input

---

In SIGNAL mode, the unit can be set to collect fractions based on an external signal (I/O).

### 4.3.1 Setting SIGNAL parameters

You program a SIGNAL mode collection run by assigning values to the input parameters shown in the table below. You can enter and save up to three sets of parameters.

**Table 4-4: SIGNAL mode input parameters**

Parameter	Default	Range	Description
parameter No.	1	1 to 3	Identifies the set of input parameters.
signal.fraction	10 counts/tube	1 to 9999 counts/tube	Number of signal counts per collection vessel.
fractionating No.	1	1 to 384, depending on rack selected	Number of samples you want to collect in the run. Entering 0 for the fractionating number programs the unit to begin collecting again at position 1 after filling all collection vessels in the rack. The unit repeats the cycle until an end signal is given.
wait.time	0	0 to 99 min, 59 sec	Time interval between the start signal and the beginning of sample collection in the first collection vessel.
multi.sample	SINGLE	SINGLE or MULTI	Allows collection of multiple runs with the same parameters in one rack, such as when using an autosampler.

**To enter the SIGNAL mode input parameters:**

1. On the MAIN MENU, select **SIGNAL** and press **ENTER**. The SIGNAL MODE menu appears.
2. Move the cursor to the [ ] to the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The current values for the selected parameter set are displayed and the cursor moves to the signal.fraction value.
4. Use the **UP** and **DOWN** keys to enter the desired signal.fraction value and press **ENTER**. The cursor moves to the fractionating No. value.
5. Use the **UP** and **DOWN** keys to enter the desired fractionating No. value and press **ENTER**. The cursor moves to the wait.time value.
6. Use the **UP** and **DOWN** keys to enter the desired wait.time value and press **ENTER**. The cursor moves to the multi sample option.



7. Use the ← and → keys to select the desired value and press **ENTER**. The cursor moves to the top of the SIGNAL MODE menu and the status changes to READY.

Once you have set the input parameters, you can start the collection run, enter another set of parameters, or return to the MAIN MENU.

### 4.3.2 Running the program

Once you have set the input parameters, you collect samples by running the program.

#### To run a SIGNAL mode program:

1. Set the SIGNAL mode input parameters as desired.
2. On the SIGNAL MODE menu, verify that the status is READY. If SET appears instead of READY, press **ESCAPE**. The status changes to READY.
3. Press **START** or send the unit an external start command.

Once the program begins, the following occurs:

- When the program begins its cycle, READY changes to RUN.
- The cursor blinks in the upper-left corner of the LCD.
- Wait.time counts down to 0, then changes to elapsed.time as the program progresses.
- Signal.fraction counts down. When the counter reaches 0, the dropper assembly moves to the next collection vessel.
- With each consecutive sampling, the fractionate ratio changes accordingly.
- Sample collection finishes when the final fraction is collected, you press END, or when you input an external end signal. When the program ends, the SIGNAL menu reappears.

After the run is complete the WFC III can display a table containing the run results. See ["Displaying the results of a run" on page 104](#), to learn how to display run results.

#### 4.3.2.1 Multiple sample runs

When you run a program in multi-sample mode, a menu similar to the one shown in the [figure "Multi-sample run display" on page 66](#) is displayed upon completion of each sampling run.

To stop sample collection and end the program, press **END**, input **EXT.END**, or use an external command.

To continue the program with the next sample run, press **START**, input **EXT. START**, or use an external command. The program then counts down the preset wait time and begins sampling again from the next collection vessel. If the skip option has been selected, the program bypasses one collection vessel, then begins collection.

**Tip:** The skip option is available only when the multi-sample mode is selected.

### 4.3.2.2 Pausing the program

You can temporarily stop a running SIGNAL mode program. To temporarily stop the program, press **PAUSE**. While the program is paused:

- Elapsed time continues to run.
- The dropper assembly moves to the drain position. If a 3-way valve is installed you can choose to have the dropper assembly pause over the current collection vessel instead of the drain. You select this option in CONDITIONS mode.
- The signal count for each sampling (signal.fraction) and the number of collection vessels (fractionating No.) can be changed.

To restart a paused program, press **START** or send an external start command. The unit resumes collection with the next collection vessel in the preset configuration.

To resume a paused program in multi-sample mode, press **START** to resume the same run (set of fractions) or press **END**, then press **START** to start a new run (set of fractions).

### 4.3.3 Sample SIGNAL mode program

You can familiarize yourself with the SIGNAL mode by entering and running the sample program provided below. The table below shows input parameter values for the sample program.

**Table 4-5: SIGNAL mode sample program parameters**

Parameter	Value
signal.fraction	3 counts/tube
fractionating No.	20
wait.time	0 sec
multi sample	SINGLE

When you run the sample program, the dropper assembly immediately moves to the first position. When an external signal is received, sample is collected into the first vessel. The dropper assembly remains over the first vessel and collects fractions when the second and third signals are received. The dropper assembly then moves to the second vessel and collects fractions at the next three signals. Fractions are collected in the same manner for the remaining 18 vessels. After the last fraction is collected in vessel number 20, the dropper assembly returns to the ready position and the run ends.

After the wait time has elapsed and the program begins, a screen similar to the example shown in the [figure "Sample program run display" on page 67](#) is displayed. Elapsed time, signal.fraction, and fractionate values change as the program progresses.

#### To set up the sample program:

1. On the MAIN MENU, select **SIGNAL** and press **ENTER**. The SIGNAL MODE menu appears on the LCD.

2. Move the cursor to the [ ] at the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The cursor moves to signal.fraction.
4. Use the **UP** and **DOWN** keys to enter **3** as the signal.fraction value and press **ENTER**. The cursor moves to fractionating No.
5. Use the **UP** and **DOWN** keys to enter **20** as the fractionating No. value and press **ENTER**. The cursor moves to wait.time.
6. Use the **UP** and **DOWN** keys to enter **0 min 0 sec.** as the wait.time value. Press **ENTER** to confirm input and move the cursor to multi sample.
7. Use the → key to select **SINGLE** and press **ENTER**. The cursor moves to the top of the SIGNAL MODE menu.
8. Move the cursor to **[to MENU]** and press **ENTER**. The MAIN MENU reappears.

The sample program is now ready to run. Follow the instructions in ["Running the program" on page 73](#), to run the program.

## 4.4 Collecting using volume

---

VOLUME mode instructs the unit to collect fractions based on a preset flow rate. The default flow rate is 10.0 mL/min and can be changed in CONDITIONS mode. Because VOLUME mode is dependent on consistency within the run, it is not appropriate for runs with a solvent gradient or change of buffer which would affect flow rate, or when the temperature fluctuates dramatically.

Changes in temperature, solution viscosity, and other environmental conditions may affect the volume of drops and the flow rate.

### 4.4.1 Setting VOLUME parameters

You program a VOLUME mode collection run by assigning values to the input parameters shown in the table below. You can enter and save up to three sets of parameters.

**Table 4–6: VOLUME mode input parameters**

Parameter	Default	Range	Description
parameter No.	1	1 to 3	Identifies the set of input parameters.
volume.fraction	10 mL/tube	0.1 to 999.9 mL/tube	Amount of sample collected per collection vessel.

**Table 4–6: VOLUME mode input parameters (continued)**

Parameter	Default	Range	Description
fractionating No.	1	1 to 384, depending on rack selected	Number of samples you want to collect in the run. Entering 0 for the fractionating number programs the unit to begin collecting again at position 1 after filling all collection vessels in the rack. The unit repeats the cycle until an end signal is given.
wait.time	0	0 to 99 min, 59 sec	Time interval between the start signal and the beginning of sample collection in the first collection vessel.
multi.sample	SINGLE	SINGLE or MULTI	Allows collection of multiple runs with the same parameters in one rack, such as when using an autosampler.

**To enter the VOLUME mode input parameters:**

1. On the MAIN MENU, select **VOLUME** and press **ENTER**. The VOLUME MODE menu appears.
2. Move the cursor to the [ ] to the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The current values for the selected parameter set are displayed and the cursor moves to the volume.fraction value.
4. Use the **UP** and **DOWN** keys to enter the desired volume.fraction value and press **ENTER**. The cursor moves to the fractionating No. value.
5. Use the **UP** and **DOWN** keys to enter the desired fractionating No. value and press **ENTER**. The cursor moves to the wait.time value.
6. Use the **UP** and **DOWN** keys to enter the desired wait.time value and press **ENTER**. The cursor moves to the multi sample option.
7. Use the ← and → keys to select the desired value and press **ENTER**. The cursor moves to the top of the VOLUME MODE menu and the status changes to READY.

Once you have set the input parameters, you can start the collection run, enter another set of parameters, or return to the MAIN MENU.

## 4.4.2 Running the program

Once you have set the input parameters, you collect samples by running the program.

### To run a **VOLUME** mode program:

1. Set the VOLUME mode input parameters as desired.
2. On the VOLUME MODE menu, verify that the status is READY. If SET appears instead of READY, press **ESCAPE**. The status changes to READY.
3. Press **START** or send the unit an external start command.

Once the program begins, the following occurs:

- When the program begins its cycle, READY changes to RUN.
- The cursor blinks in the upper-left corner of the LCD.
- Wait.time counts down to 0, then changes to elapsed.time as the program progresses.
- Volume.fraction counts down. When the counter reaches 0, the dropper assembly moves to the next collection vessel.
- With each consecutive sampling, the fractionate ratio changes accordingly.
- Sample collection finishes when the final fraction is collected, you press END, or when you input an external end signal. When the program ends, the VOLUME MODE menu reappears.

After the run is complete the WFC III can display a table containing the run results. See ["Displaying the results of a run" on page 104](#), to learn how to display run results.

#### 4.4.2.1 Multiple sample runs

When you run a program in multi-sample mode, a menu similar to the one shown in the [figure "Multi-sample run display" on page 66](#) is displayed upon completion of each sampling run.

To stop sample collection and end the program, press **END**, input **EXT.END**, or use an external command.

To continue the program with the next sample run, press **START**, input **EXT. START**, or use an external command. The program then counts down the preset wait time and begins sampling again from the next collection vessel. If the skip option has been selected, the program bypasses one collection vessel, then begins collection.

**Tip:** The skip option is available only when the multi-sample mode is selected.

#### 4.4.2.2 Pausing the program

You can temporarily stop a running VOLUME mode program. To temporarily stop the program, press **PAUSE**. While the program is paused:

- Elapsed time continues to run.
- The dropper assembly moves to the drain position. If a 3-way valve is installed you can choose to have the dropper assembly pause over the current collection vessel instead of the drain. You select this option in CONDITIONS mode.
- The volume for each sampling (volume.fraction) and the number of collection vessels (fractionating No.) can be changed.

To restart a paused program, press **START** or send an external start command. The unit resumes collection with the next collection vessel in the preset configuration.

To resume a paused program in multi-sample mode, press **START** to resume the same run (set of fractions) or press **END**, then press **START** to start a new run (set of fractions).

### 4.4.3 Sample VOLUME mode program

You can familiarize yourself with the VOLUME mode by entering and running the sample program provided below. The table below shows the input parameter values for the sample program.

**Table 4–7: VOLUME mode sample program parameters**

Parameter	Value
volume.fraction	42.5 mL/tube
fractionating No.	10
wait.time	15 sec
multi sample	MULTI
skip	ON

When you run the sample program, the dropper assembly holds in the ready position over the drain for 15 seconds before it moves to collect 42.5-mL fractions in collection vessels 1 through 10. The dropper assembly then returns to the ready position. When **START** is pressed, the dropper assembly again holds for 15 seconds in the ready position, then skips collection vessel 11 before collecting 10 more 42.5-mL fractions in collection vessels 12 through 21. The dropper assembly then returns to the ready position and the run ends.

After the wait time has elapsed and the program begins, a screen similar to the example shown in the [figure "Sample program run display" on page 67](#) is displayed. Elapsed time, volume.fraction, and fractionate values change as the program progresses.

#### To set up the sample program:

1. On the MAIN MENU, select **VOLUME** and press **ENTER**. The VOLUME MODE menu appears on the LCD.
2. Move the cursor to the [ ] at the right of parameter No.
3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The cursor moves to volume.fraction.
4. Use the **UP** and **DOWN** keys to enter **42.5** as the volume.fraction value and press **ENTER**. The cursor moves to fractionating No.
5. Use the **UP** and **DOWN** keys to enter **10** as the fractionating No. value and press **ENTER**. The cursor moves to wait.time.
6. Use the **UP** and **DOWN** keys to enter **0 min 15 sec.** as the wait.time value. Press **ENTER** to confirm input and move the cursor to multi sample.

7. Use the → key to select **MULTI** and press **ENTER**. The cursor moves to the top of the VOLUME MODE menu.
8. Move the cursor to **[to MENU]** and press **ENTER**. The MAIN MENU reappears.
9. Move the cursor to **CONDITIONS** and press **ENTER**. The CONDITIONS menu appears.
10. Move the cursor to **multi.s skip [ON]** and press **ENTER**.
11. Move the cursor to **[to MENU]** and press **ENTER**. The MAIN MENU reappears.

The sample program is now ready to run. Follow the instructions in ["Running the program" on page 76](#), to run the program.





# 5 Collecting Fractions Using Advanced Modes

This chapter describes how to use the Waters Fraction Collector III's (WFC III) advanced modes to collect fractions. Advanced modes, which are described in ["Collecting using peak discrimination" on page 81](#) to ["Collecting using sampling intervals" on page 100](#), include:

- PEAK
- WINDOW
- MANUAL
- SAMPLING

## 5.1 Collecting using peak discrimination

---

The WFC III includes a peak discriminator that examines peak signals sent from chromatography systems or other detectors. The peak discriminator can be programmed to collect fractions based on four discrimination criteria:

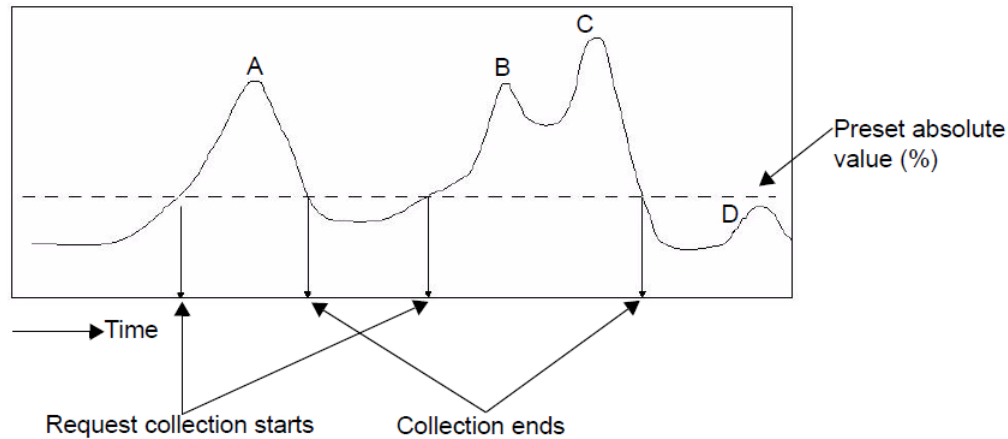
- Absolute value
- Slope value
- Absolute and slope value
- Peak number

### 5.1.0.1 Absolute value discrimination

When you choose absolute value as the discrimination criteria, only peak data greater than a preset percentage of the signal level is collected. Collection begins when the level rises above the preset value and continues until the level falls below the preset value.

In the example shown below, peaks A, B, and C are collected. Peaks B and C are not distinguished because the detection level does not fall below the preset absolute value between the two peaks. Peak D is not collected because the signal does not exceed the preset absolute value.

**Figure 5-1: Absolute value discrimination**

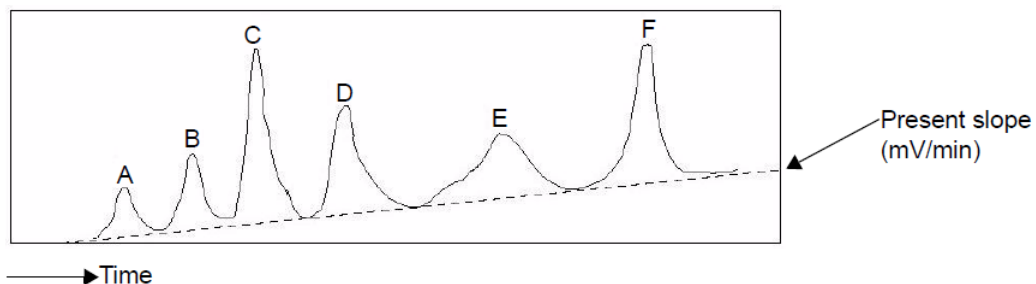


### 5.1.0.2 Slope value discrimination

When you choose slope value as the discrimination criteria, data above the preset slope value is collected. Collection begins when the slope of the signal exceeds the preset value and continues until the signal slope falls below the preset value. Slope value discrimination should be used when there is a large drift in the baseline and/or the peaks are sharp.

In the example shown below, peaks A through F are detected.

**Figure 5-2: Slope value discrimination**

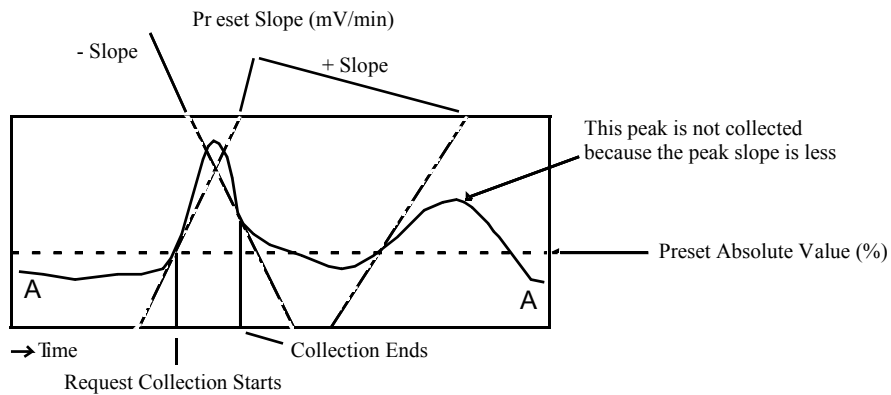


### 5.1.0.3 Absolute and slope value discrimination

You can also collect fractions based on both absolute and slope values. In order for collection to occur, both conditions must be satisfied. This setting is useful for eliminating incidental collection resulting from a noisy baseline.

In the example shown below, both peaks exceed the preset absolute value. However, only the first peak is collected because its slope value exceeds the preset slope value.

**Figure 5-3: Absolute and slope value discrimination**

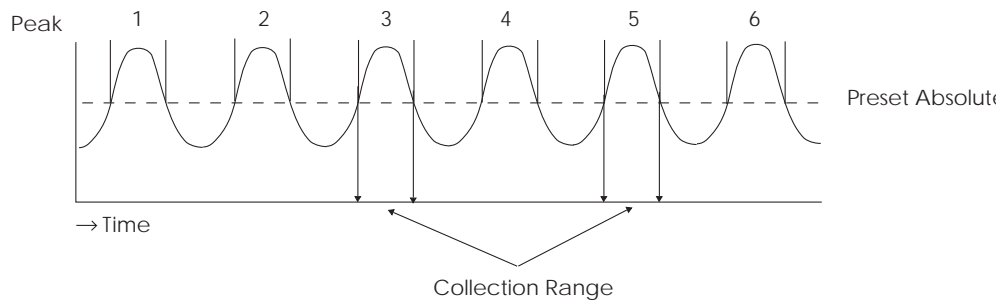


#### 5.1.0.4 Peak number discrimination

Peak number discrimination allows you to detect up to 99 peaks and designate up to 10 of those peaks for collection. You program an absolute value above which peaks are detected and specify which peaks you want to collect. The WFC III numbers the peaks beginning with the first peak detected following the end of wait time and collects the peaks you preselected.

In the example show below, six peaks are detected. Only peaks 3 and 5 are designated for collection.

**Figure 5-4: Peak number discrimination**



### 5.1.1 Setting PEAK parameters

You program a PEAK mode collection run by assigning values to the input parameters. Setting PEAK mode parameters involves three steps:

1. Set general parameters.
2. Set advanced parameters.
3. Set peak numbers (if you are using peak number discrimination).

### 5.1.1.1 Setting general parameters

PEAK mode general parameters include time values for the run as well as the multi-sample option. You set the general parameters on the PEAK MODE menu. You can enter and store up to three sets of parameters. The table below shows the general PEAK mode parameters.

**Table 5–1: PEAK mode general parameters**

Parameter	Default	Range	Description
parameter No.	1	1 to 3	Identifies the set of input parameters.
wait.time	0	0 to 99 min, 59 sec	Initial wait time before beginning peak detection. Presetting a wait time longer than the end time automatically renews the end time.
end.time	10 min.	1 to 999 min.	End of peak detection and collection time. This value cannot be less than that allotted for the wait time.
request.time	10 sec.	0 to 99 min, 59 sec	Collection time per collection vessel when all selected peak criteria have been met (peak samples). If this time is not set, the sample is directed to drain.
monitor.time	0 sec.	0 to 99 min, 59 sec	Time per collection vessel when the sample does not meet selected peak criteria. If this time is not set, the nonpeak sample is directed to drain.
multi sample	SINGLE	SINGLE or MULTI	Activates the multi-sample option if you want to collect multiple runs with the same parameters in one rack, such as when using an autosampler. To activate, move the cursor from <b>[SINGLE]</b> to <b>[MULTI]</b> and press <b>ENTER</b> . An asterisk (*) appears next to the selection.

**To set the PEAK mode general parameters:**

1. On the MAIN MENU, select **PEAK** and press **ENTER**. The PEAK MODE menu appears.
2. Move the cursor to the **[ ]** to the right of parameter No.

3. Use the **UP** and **DOWN** keys to enter the parameter No. and press **ENTER**. The current values for the selected parameter set are displayed and the cursor moves to the wait time value.
4. Use the **UP** and **DOWN** keys to enter the desired wait time and press **ENTER** to confirm your input. The cursor moves to the end time value.
5. Use the **UP** and **DOWN** keys to enter the desired end time and press **ENTER** to confirm your input. The cursor moves to the request time value.
6. Use the **UP** and **DOWN** keys to enter the desired request time and press **ENTER** to confirm your input. The cursor moves to the monitor time value.
7. Use the **UP** and **DOWN** keys to enter the desired monitor time and press **ENTER** to confirm your input. The cursor moves to the multi sample option.
8. Use the ← and → keys to select the multi sample option and press **ENTER**. The cursor moves to [SET PEAK NO.].
9. Select [**to MENU**] and press **ENTER** to return to the MAIN MENU or select [**PEAK PARA**] and press **ENTER** to set PEAK mode advanced parameters.

**Tip:** If the WFC III loses power while the peak parameters screen is displayed or while the unit is in RUN or NEXT status, the unit will return to the peak parameters screen when power is restored.

### 5.1.1.2 Setting advanced parameters

PEAK mode advanced parameters specify the criteria for sample collection in PEAK mode. You set the advanced parameters on the PEAK PARAMETER menu. The table below shows the PEAK mode advanced parameters.

**Table 5–2: PEAK mode advanced parameters**

Parameter	Default	Range	Description
peak.level (absolute value)	0	0 to 100% of input (10 mV or 1 V full scale - see CONDITIONS mode)	Determines when fraction collection begins and ends. When this parameter is set to 0, slope value acts as the sole discriminator.
base.drift max (slope value)	0	0 to 99.99 mV/min	Signal level change (gradient) of the input signal expressed in mV/min. When the rate of change exceeds this value, collection begins.

**Table 5–2: PEAK mode advanced parameters (continued)**

<b>Parameter</b>	<b>Default</b>	<b>Range</b>	<b>Description</b>
signal sampling	LOW	LOW or HIGH	Speed at which the chromatogram signal is read. At the HIGH setting, the signal is read every 0.5 seconds. At the LOW setting, the signal is read every 3 seconds. A setting of HIGH is recommended for HPLC systems and LOW is recommended for LPLC systems.
delaying time	0	0 to 9 min, 59 sec	Preset if the distance from the detector to the dropper assembly of the Fraction Collector III is long and/or the flow rate is slow. See <a href="#">page 86</a> .

**To set the PEAK mode advanced parameters:**

1. On the PEAK MODE menu, select **PEAK PARA** and press **ENTER**. The PEAK PARAMETER menu appears.
2. If you want to collect fractions based on absolute value, absolute and slope values, or peak numbers, move the cursor to **peak.level** and use the **UP** and **DOWN** keys to set the peak level (absolute value).
3. Press **ENTER**. The cursor moves to base.drift max.
4. If you want to collect fractions based on slope value or absolute and slope values, use the **UP** and **DOWN** keys to set the base.drift max (slope value) and press **ENTER**. The cursor moves to the signal sampling option.
5. Use the ← and → keys to select the signal sampling rate and press **ENTER**. The cursor moves to the delaying time value.
6. Use the **UP** and **DOWN** keys to set the delaying time and press **ENTER**. The cursor moves to PEAK MODE.
7. Press **ENTER** to return to the PEAK MODE menu or select **[to MENU]** and press **ENTER** to return to the MAIN MENU.

**5.1.1.3 Calculating delay time**

If the distance from the peak detector to the dropper assembly is long and/or the flow rate is slow, you should preset delay time. Delay time compensates for the time it

takes sample to travel through the tubing to the dropper assembly. Setting the correct delay time ensures that the sample collected is actually from the peak.

**Table 5-3: To determine the appropriate delay time, use the following formula**

Transport tube capacity between the peak detector and the dropper assembly (mL)	= Delay Time (min)
Flow Rate (mL/min)	

The transport tube capacity between the peak detector and the dropper assembly can be determined experimentally or by calculation. To calculate the transport tube capacity, use the following formula:

$$\text{Volume (ml)} = ((\text{internal diameter in cm})/2)^2 \times \pi \times \text{length of tubing in cm}$$

**Table 5-4: For a 10-cm length of 1.0-mm ID tubing, the volume calculation is as follows**

Volume	= $(0.1 \text{ cm}/2)^2 \times \pi \times 10 \text{ cm}$
	= $0.0025 \text{ cm}^2 \times 3.14159 \times 10 \text{ cm}$
	= $0.0785 \text{ cm}^3$
	= 0.0785 ml or 78.5 $\mu\text{l}$

#### 5.1.1.4 Setting peak numbers

If you are using peak number discrimination, you must specify which peaks you want to collect. You do this by setting the peak numbers. Each peak number you set should be smaller than the next peak number and larger than the previous peak number.

##### To set peak numbers:

1. On the PEAK MODE menu, move the cursor to **SET PEAK No.** and press **ENTER**. The Set Peak Numbers screen appears.
2. Move the cursor to **Peak No. 1 [ ]** and use the **UP** and **DOWN** keys to set the peak number.
3. Press **ENTER** to confirm (or **ESCAPE** to cancel your entry). The cursor moves to the next peak number.
4. Continue entering peak numbers until you have set the desired peaks.
5. When you have set the desired peak numbers, press **END**. The cursor moves to the first peak number.
6. Move the cursor to **PEAK MODE** and press **ENTER** to return to the PEAK MODE menu, or select **[to MENU]** and press **ENTER** to return to the MAIN MENU.

## 5.1.2 Running the program

Once you set the PEAK mode parameters, you can run the program and collect samples. Note that once you begin a program in PEAK mode, it cannot be paused.

### To run a PEAK mode program:

1. Set the PEAK mode input parameters as desired.
2. With the PEAK MODE menu displayed, press **START** or send the unit an external start command.

Once the program begins, the following occurs:

- **wait.time** counts down to 0, then changes to **elapsed.time** as the program progresses. The run length (end time – start time) is displayed to the right of the elapsed time.
- **peak No.** indicates the current peak being collected followed by the total number of preset peaks to be collected.
- **s.** displays the slope direction using arrows and the slope value in voltage units.
- **request** indicates that sample fractions are being collected which meet the selected criteria (absolute value, slope value, peak number, or absolute and slope value); **monitor** indicates that the fractions being collected do not meet selected criteria (not peak or not selected peak).
- **p.ON** indicates the unit is in request mode; **p.OFF** indicates the unit is in monitor or drain mode. Peak value is displayed as % of full scale.
- **fractionate** displays the number of the current sample being collected followed by the sample number currently being monitored.
- When the multi-sample option is selected, multi sample is displayed followed by the run number.
- Sample collection finishes when the END time is reached, END is pressed, EXT.END is input, or when an external end command is sent to the unit.

If the multi-sample option is selected, the screen displays NEXT after the completion of each run and waits for a restart command.

### 5.1.2.1 Displaying run results

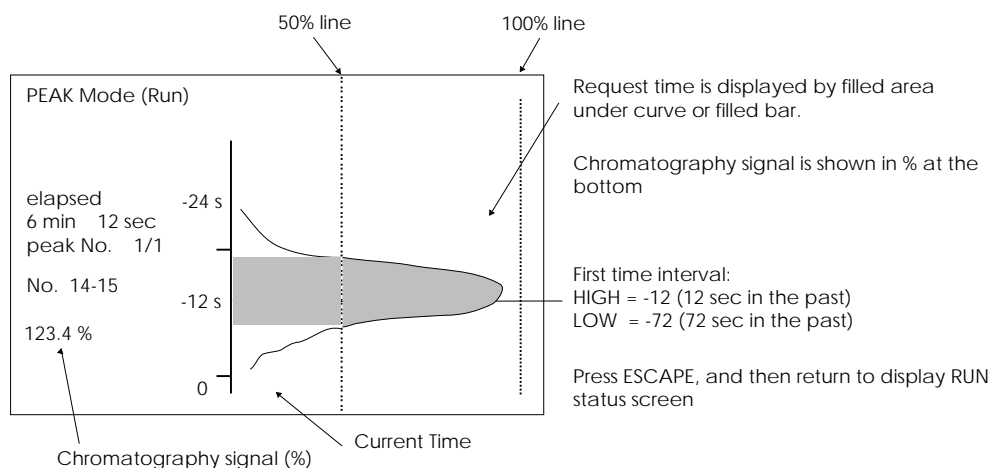
You can display a chromatogram while the run is in progress.

#### To display a chromatogram while the run is in progress:

1. Start the program.
2. At any time after wait time has elapsed, press **ENTER**. The chromatogram appears. The figure below shows a sample PEAK mode chromatogram.



**Figure 5–5: Sample PEAK mode chromatogram**



You can also view collection results after the run is complete. The results can be viewed as a table (showing fraction number, event trigger, start time, and lap time values) or as a chromatogram. For more information about viewing run results, see ["Displaying the results of a run" on page 104](#).

### 5.1.3 Sample PEAK mode program

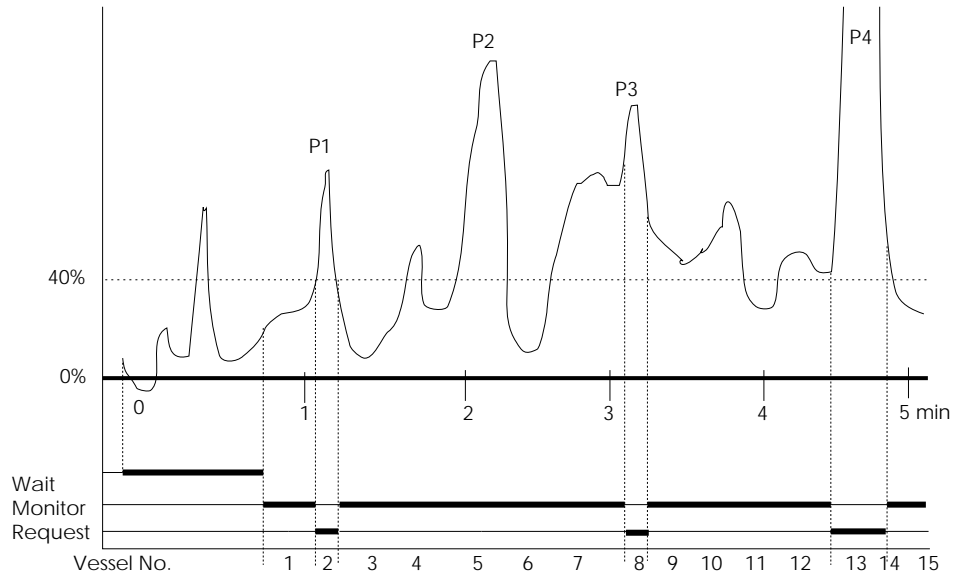
You can familiarize yourself with the PEAK mode by entering and running the sample program provided below. The table below shows the parameter values for the sample PEAK mode program.

**Table 5–5: PEAK mode sample program parameters**

Parameter	Value
<b>General parameters</b>	
wait time	40 sec
end time	6 min
request time	12 sec
monitor time	24 sec
<b>Advanced parameters</b>	
absolute value	40%
slope level	20 mV/min
response	HIGH
delay time	0 sec
<b>Peak numbers</b>	
peak No. 1	1
peak No. 2	3
peak No. 3	4

When you run the sample program, the WFC III waits for 40 seconds before monitoring for peaks above 40% with a slope greater than 20 mV/min. It collects peak number 1, 3, and 4 for 12 seconds and then ends after 6 minutes. The figure below displays a chromatogram from the sample program.

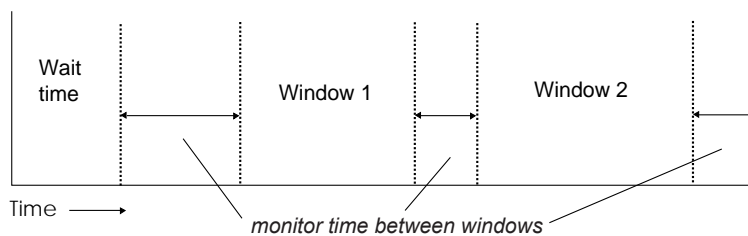
**Figure 5-6: Sample program chromatogram**



## 5.2 Collecting using time windows

The WINDOW mode allows you to define a set period of time (window) during which peaks can be detected and sample collected. You can define up to 10 windows with distinct peak selection criteria (absolute value and slope value) for each window. The figure below shows a graphic representation of window collection.

**Figure 5-7: Window collection**



### 5.2.1 Setting WINDOW parameters

You program a WINDOW mode collection run by assigning values to the input parameters.

### To set WINDOW mode parameters:

1. Set general parameters.
2. Set advanced parameters.
3. Set peak parameters.

#### 5.2.1.1 Setting general parameters

WINDOW mode general parameters include the time values for the run as well as the multi-sample option. You set the general parameters on the WINDOW MODE menu. You can enter and store up to three sets of parameters.

The table below shows the WINDOW mode general parameters.

**Table 5-6: WINDOW mode general parameters**

Parameter	Default	Range	Description
parameter No.	1	1 to 3	Identifies the set of input parameters.
wait.time	0	0 to 99 min, 59 sec	Time interval between the start signal and beginning collection in the first collection vessel. Presetting a wait time longer than the end time automatically renews the end time.
multi sample	SINGLE	SINGLE or MULTI	Activates the multi-sample option if you want to collect multiple runs with the same parameters in one rack, such as when using an autosampler. To activate, move the cursor from [SINGLE] to [MULTI] and press <b>ENTER</b> . An asterisk (*) appears next to the selection.
between window monitor.time	0	0 to 99 min, 59 sec	Collection time per collection vessel between windows. Setting this parameter to 0 sends all between-window sample to drain.

Once you have preset these parameters, move the cursor to **SET WINDOW** and press **ENTER** to display the SET WINDOW menu.

#### 5.2.1.2 Setting advanced parameters

WINDOW mode advanced parameters define the collection windows. You set the advanced parameters on the SET WINDOW menu. You can define up to 10 windows for each set of input parameters. The figure below shows the SET WINDOW menu.

**Figure 5-8: SET WINDOW menu**

```

<SET WINDOW> ----WINDOW MODE (PN1)----
  01 : start [ 3 m 45 s] end [ 8m 45 s]¶
        request [ 0 m 20 s] monitor[ ]¶
  02 : start [15 m 10 s] end [18m 45 s]¶
        request [ 0 m 50 s] monitor [ 0m
34s]¶
[03 - 04] [09 -10 ] [PEAK PARA.] [to
MENU]¶
$

```

The table below shows the WINDOW mode advanced parameters.

**Table 5-7: WINDOW mode advanced parameters**

Parameter	Default	Range	Description
start	0	1 sec to 999 min, 58 sec	Start time should be longer than the previous window's end time and shorter than the end time for the current window.
end	0	1 sec to 999 min, 59 sec	End time should be longer than the current window's start time and shorter than the next start time.
request	0	0 to 99 min, 59 sec	Collection time per collection vessel when all selected peak criteria have been met (peak samples). If request time is not set, the sample is directed to drain.
monitor	0	0 to 99 min, 59 sec	Time per collection vessel when the sample does not meet selected peak criteria. If this time is not set, the nonpeak sample is directed to drain.

**To set the WINDOW mode advanced parameters:**

1. On the WINDOW MODE menu, move the cursor to **SET WINDOW** and press **ENTER**. The SET WINDOW menu appears.
2. Move the cursor to **start [ ]** and use the **UP** and **DOWN** keys to enter the start time for the first window. You cannot enter a start time value less than the wait time you selected on the WINDOW MODE menu.
3. Press **ENTER**. The cursor moves to end time.

4. Use the **UP** and **DOWN** keys to enter the end time for the first window. The end time value cannot be less than the start time value.
5. Press **ENTER**. The cursor moves to request time.
6. Use the **UP** and **DOWN** keys to enter the request time and press **ENTER**. The cursor moves to monitor time.
7. Use the **UP** and **DOWN** keys to enter the monitor time and press **ENTER**. The cursor moves to the parameter set for the second window.
8. Follow steps 2 through 7 to define the second window.
9. If you want to define more than two windows, move the cursor to **[03 - 04]** and press **ENTER**. The parameter sets for the third and fourth windows appear.
10. Follow steps 2 through 9 to define the third and fourth (and any subsequent) windows.
11. When you are finished setting the window parameters, move the cursor to **[WIND. MODE]** and press **ENTER**. The WINDOW MODE menu reappears.

**Tip:** You must enter all parameter values for a window before you can set the parameters for the next window.

Press **END** while the cursor is on a [ ] within a window to clear data from that window and all subsequent windows.

### 5.2.1.3 Setting PEAK parameters

Once you have defined the time windows for sample collection, you must specify the criteria used for collection. You use the PEAK MODE menu to define the collection criteria. Absolute value and slope value can be used alone or in combination to determine the peaks to be collected within the specified windows. Setting absolute value and slope value to 0 requests all sample to be collected for each window.

See ["Collecting using peak discrimination" on page 81](#), for details on setting peak parameters. After you set the peak parameters, return to the WINDOW MODE menu.

## 5.2.2 Running the program

Once you set the WINDOW mode parameters, you can run the program and collect samples. Note that once you begin a program in WINDOW mode, it cannot be paused. When the program is running, the screen shown in the [figure "WINDOW MODE run display" on page 94](#) is displayed.

### To run a WINDOW mode program:

1. Set the WINDOW mode input parameters as desired.
2. On the WINDOW MODE menu, press **START** or send the unit an external start command.

**Figure 5–9: WINDOW MODE run display**

```
----- WINDOW MODE ----- (RUN) STANDARD¶
wait. time      : 2 min 12sec¶
window. time    : 95 min 12sec - 123min
45sec¶
s. -->
9.3 mV ¶
request         : 12 min 12 sec p. OFF
93.4%¶
fractionate     : 18 - 23 multi sample
1¶
$
```

Once the program begins, the following occurs:

- **wait.time** counts down to 0, then changes to **elapsed.time** as the program progresses (shown as elapsed time/end time of final window in minutes and seconds).
- **window.time** displays the current window's start and end times. During wait time and between windows, **window.time** displays the next window's start and end times.
- **s.** displays slope direction using arrows and slope value in voltage units.
- **request** indicates that sample fractions are being collected within the windows which meet the selected criteria (absolute value and slope value); **monitor** indicates that the fractions being collected do not meet selected criteria (nonpeak or unselected peak). Between specified time windows the display will read between.win.
- **p.ON** indicates the unit is in request mode; **p.OFF** indicates the unit is in monitor or drain mode. Peak value is displayed as % of full scale.
- **fractionate** displays the sample currently being collected followed by the sample number currently being monitored.
- When the multi-sample option is selected, multi sample is displayed followed by the run number.
- Sample collection finishes when the END time is reached, you press END, EXT.END is input, or an external end command is sent to the unit.

If the multi-sample option is selected, the screen displays NEXT at the end of each run and a START signal must be received to begin the next run. An END signal received in this mode terminates the program.

### 5.2.2.1 Displaying run results

You can display a chromatogram while the run is in progress.

### To display a chromatogram while the run is in progress:

1. Start the program.
2. At any time after wait time has elapsed, press **ENTER**. The chromatogram appears.

You can also view collection results after the run is complete. The results can be viewed as a table (showing fraction number, event trigger, start time, and lap time values) or as a chromatogram. For more information about viewing run results, see ["Displaying the results of a run" on page 104](#).

## 5.2.3 Sample WINDOW mode program

You can familiarize yourself with the WINDOW mode by entering and running the sample program provided below. The table below shows the parameter values for the sample WINDOW mode program.

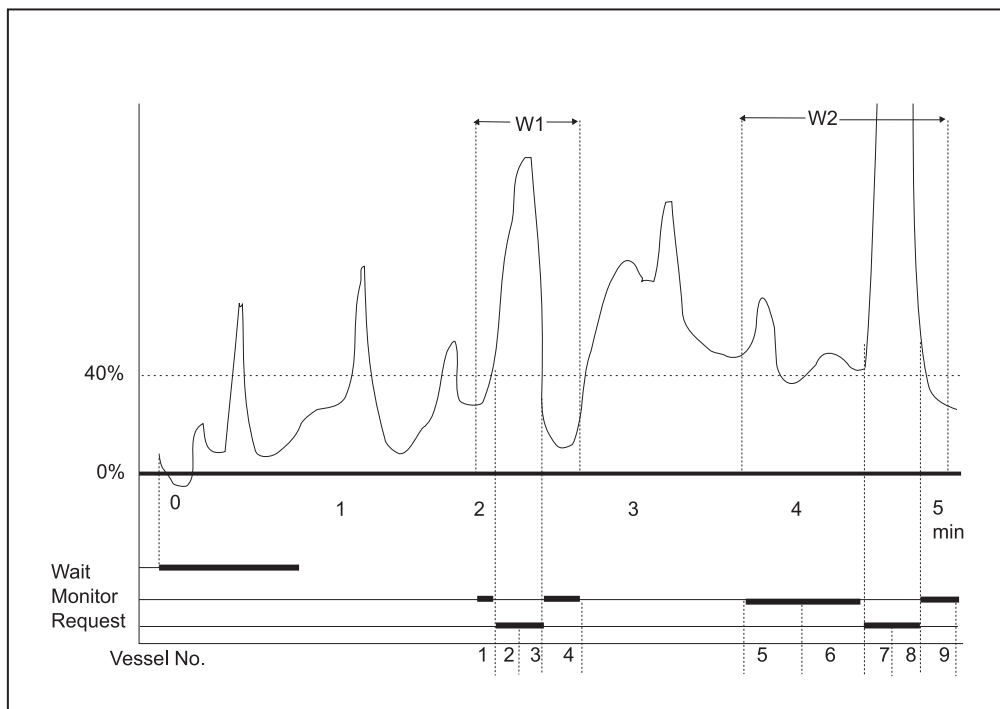
**Table 5–8: WINDOW mode sample program parameters**

Parameter	Value
<b>General parameters</b>	
wait time	40 sec
between window monitor.time	0 sec
<b>Advanced parameters</b>	
window 1	Start: 2 min 00 sec End: 2 min 32 sec Request: 08 sec Monitor: 24 sec
window 2	Start: 3 min 32 sec End: 5 min 00 sec Request: 08 sec Monitor 24 sec
<b>Peak parameters</b>	
absolute value	40%
slope level	20 mV/min
response	HIGH
delay time	0 sec

When you run the sample program, the program waits for 40 seconds. After 2 minutes have elapsed, the first window begins. During the window, the peak discriminator monitors for peaks above 40% with a slope greater than 20 mV/min. If this criteria is met during the window, sample is collected for 8 seconds. When 2 minutes and 32 seconds have elapsed, window 1 ends. At 3 minutes, 32 seconds, the second window begins and the peak discriminator monitors for peaks using the same criteria as the first window. After 5 minutes have elapsed, the second window ends and the run is complete.

The figure below shows a chromatogram of the sample program.

**Figure 5–10: Sample WINDOW mode chromatogram**



### **5.3 Collecting using a programmed sequence**

The MANUAL mode has two functions, TEACHING and PLAYBACK. You use the TEACHING function to teach a series of commands to the WFC III by pressing the panel keys to control the movement of the dropper assembly. You use the PLAYBACK function to replay the sequence of commands. This can be useful when direct connection of a detector to the WFC III is not practical or when the PEAK or WINDOW modes are not suitable.

The figure below shows the MANUAL MODE menu.



**Figure 5–11: MANUAL MODE menu**

```

---MANUAL MODE ---(TEACHING) STANDARD¶
End Key      : stop [*inst'ly] [with delay]¶
delay.time   : [12 min 34 sec] graph [*L]
              [H]¶
Key function after start key pushed¶
    UP = Move to next tube with delay¶
    PAUSE = Move to a drain with delay¶
    END  = finish sequence¶
[PLAYBACK]  [to MENU]$
    
```

### 5.3.1 Setting MANUAL parameters

You use the TEACHING function to set the MANUAL mode collection parameters. The table below shows the MANUAL mode parameters.

**Table 5–9: MANUAL mode parameters**

Parameter	Default	Range	Description
end key	instantly	instantly or with delay	Choose to end the program instantly when END is selected or after the delay time entered on the line below.
delay.time	0	0 to 99 min, 59 sec	Allows for sample travel time between the signal and collection of fraction. The delay occurs with both TEACHING and PLAYBACK functions. See <a href="#">"Setting PEAK parameters" on page 83</a> , for information on calculating delay time.

During the run, a graph can be displayed by pressing ENTER after the run has started. To use this option, move the cursor to graph [L] or [H]. Response speed can be selected by choosing [L]=LOW 3 sec or [H]=HIGH 0.5 sec.

Manual collection begins when you press START, input EXT. START, or send the unit an externally generated start command.

### To program using the **TEACHING** function:

1. On the MAIN MENU, select **MANUAL** and press **ENTER**. The MANUAL MODE menu appears.

**Figure 5–12: MANUAL MODE teaching menu**

```
-----MANUAL MODE -----(TEACHING)STANDARD¶
End Key      : stop [*inst`ly] [with
delay]¶
delay.time   : [12 min 34 sec] graph [*L]
[H]¶
elapsed time : 123 min 12 sec¶
fractionate  : request No. 123 - 123$
```

2. Select **TEACHING** and press **ENTER**.
3. Press **START** to move the dropper assembly to drain position and to begin the teaching sequence.
4. Press **UP** to move the dropper assembly to the next collection vessel after delay time has elapsed. When collection into the last collection vessel has been completed, the program automatically ends.
5. Press **PAUSE** to drain after delay time has elapsed.
6. Press **END** to end collection after delay time has elapsed. After **END**, the display returns to the previous menu.

The WFC III beeps to confirm each UP, PAUSE, and END command.

Data collected in TEACHING function is saved in memory and can be repeated. Delay time can be changed before beginning playback.

Press **ENTER** while in MANUAL (TEACHING) mode to display a graph showing signal input and run parameters.

## 5.3.2 Running the program

Once you use the TEACHING function to program a collection sequence, you can use the PLAYBACK function to run the program and collect samples.

### To run a **MANUAL** mode program:

1. Use the TEACHING function to program a collection sequence.
2. On the MANUAL MODE menu, select **PLAYBACK** and press **ENTER**. The result of TEACHING appears as a table. If results use more than one screen display, you can use the **UP** and **DOWN** keys to scroll through the pages.
3. Press **START** to begin PLAYBACK. The collection sequence is executed.
4. Press **END** to terminate the run.

### 5.3.2.1 Displaying run results

Pressing **ENTER** while in PLAYBACK mode displays detector input and a bar graph of request/monitor times. The [figure "MANUAL MODE playback menu" on page 99](#) below shows the PLAYBACK function displaying the results of the TEACHING function.

**Figure 5–13: MANUAL MODE playback menu**

```

-----MANUAL
MODE----- (PLAYBACK)STANDARD
No.      EVENT      TRIG      START.T      LAP      TIME
-----
0        START      KEY              0m 50s
1        REQUEST    NORMAL 0m 50s      0m 20s
0        DRAIN      -          1m 10s      0m 20s
2        REQUEST    NORMAL 1m 30s      0m 20s
3        REQUEST    NORMAL 1m 50s      0m 20s

```

The figure below shows the PLAYBACK screen during the run.

**Figure 5–14: MANUAL MODE playback run display**

```

-----MANUAL MODE
----- (PLAYBACK)STANDARD

elapsed time   : 123min 12sec
┆
fractionate    : request No.123 - 123
┆
0  START      KEY              0m 50s
1  REQUEST    NORMAL 0m 50s      0m 20s
0  DRAIN      -          1m 10s      0m 20s

```

Time at START shows wait time at drain. For an explanation of terms and abbreviations used in the table, see ["Displaying the results of a run" on page 104](#).

## 5.4 Collecting using sampling intervals

SAMPLING mode enables you to take samples from the effluent at specified time intervals and collect repeat samples into the same collection vessel. You can control the amount of sample collected by time or by volume. The figure below shows the SAMPLING MODE menu.

**Figure 5–15: SAMPLING MODE menu**

```

-----SAMPLING MODE -----(READY) STANDARD
parameter No.      : [ 1 ]
fraction           : [*TIME] [ VOLUME]
time. fraction     : [12min 34sec]
fractionating No. : [120] tube 0 =
endless
wait. time         : [ 0min 0sec]
interval. time     : [123min 45sec]
repeat            : [12] / tube [to MENU]
  
```

### 5.4.1 Setting SAMPLING parameters

You program a SAMPLING mode collection run by assigning values to the input parameters. The table below lists the available parameters, their default values, and the range of valid values for SAMPLING mode. You can enter and store up to three sets of parameters.

**Table 5–10: SAMPLING mode parameters**

Parameter	Default	Range	Description
parameter No.	1	1 to 3	Identifies the set of input parameters.
fraction	TIME	TIME or VOLUME	Allows you to select the method of fraction collection. If you choose TIME, the time.fraction parameter appears on the next line of the menu. If you choose VOLUME, the vol.fraction parameter appears.
vol.fraction	0	0 to 999.9 ml	Presets the volume of sample collected per sampling. The volume is based on flow rate, which can be input in CONDITIONS mode (default is 10 ml/min).

**Table 5–10: SAMPLING mode parameters (continued)**

Parameter	Default	Range	Description
time.fraction	0	0 to 99 min, 59 sec	Collection time per sampling.
fractionating No.	1	0 to 384, depending on rack selected	Number of collection vessels you want to collect samples in for the run. If you enter 0, the unit begins collecting again at position 1 after filling all collection vessels in a rack and repeats the cycle until an end signal is given.
wait.time	0	0 to 99 min, 59 sec	Time between the start signal and beginning collection in the first collection vessel. Presetting a longer wait time than end time automatically renews end time.
interval.time	0	0 to 99 min, 59 sec	Time between the beginning of one fraction and the beginning of the next fraction. Between the end of the fraction (time or volume) and the end of the interval, sample is directed to drain.
repeat	1	1 to 99 times	Number of samplings per collection vessel.

**To specify SAMPLING mode parameters:**

1. On the MAIN MENU, select **SAMPLING** and press **ENTER**. The SAMPLING MODE menu appears.
2. Move the cursor to **fraction** and use the ← and → keys to choose the desired fraction type.
3. Press **ENTER** to confirm your input.
4. Set the time.fraction or vol.fraction, fractionating No., wait.time, interval.time, and repeat parameters.

**5.4.2 Running the program**

Once you set the sampling mode parameters, you can run the program and collect samples.

**To run a SAMPLING mode program:**

1. Set the SAMPLING mode input parameters as desired.
2. On the SAMPLING MODE menu, press **START** or transmit an external start signal to the WFC III.

Once the program begins, the following occurs:

- **wait.time** counts down to 0, then changes to elapsed.time as the program progresses.
- **interval.time** counts down the amount of time remaining in the interval.
- **repeat** indicates the number of samplings taken followed by the number of samplings per collection vessel.
- **time.fraction** indicates the time remaining on the current sampling (if you are collecting fractions by time). When this number reaches 0, the dropper assembly moves to drain until the end of the interval.
- **vol.fraction** indicates the amount collected into the current collection vessel and the total amount to be collected into the collection vessel (if you are collecting fractions by volume).
- **fractionate** shows the number of the current collection vessel and the total number of collection vessels (fractionating No.).
- Collection ends when the run completes, you press END, or the unit receives an external end signal.

The figure below shows an example of the LCD display during a SAMPLING mode run.

**Figure 5–16: SAMPLING MODE run display**

```

----- SAMPLING MODE ----- (RUN)
STANDARD¶

wait.time       : 0min 12sec¶

interval.time   : 12min 12sec  repeat:
1/5¶

time.fraction   : 12min 12sec ¶

fractionate     : 120/120¶

-----
--- ¶

UP   =  advance to the next point$

```

### 5.4.2.1 Pausing the program

While the program is running, you can press PAUSE to temporarily stop the program.

When the program is paused, the display is the same as in TIME and VOLUME simple modes. However, the top line reads SAMPLING MODE rather than TIME or VOLUME MODE. The figure below shows the SAMPLING MODE menu when the program is paused.

**Figure 5–17: SAMPLING MODE pause menu**

```

----- SAMPLING MODE ----- (PAUSE) STANDARD
elapsed. time      : 123 min 12sec
time. fraction     : [12 min 34 sec]/tube
fractionating No.  : [115] now No. 110
-----
--- START = restart (to the next point)
END = the stop of RUN MODES

```

While the program is paused, the time or volume for each sampling (time.fraction or vol.fraction) and the number of collection vessels (fractionating No.) can be changed.

You can resume a paused program by pressing START or by sending the unit a start command. The program resumes collection beginning with the next collection vessel (next fractionating No.).

### 5.4.3 Sample SAMPLING mode program

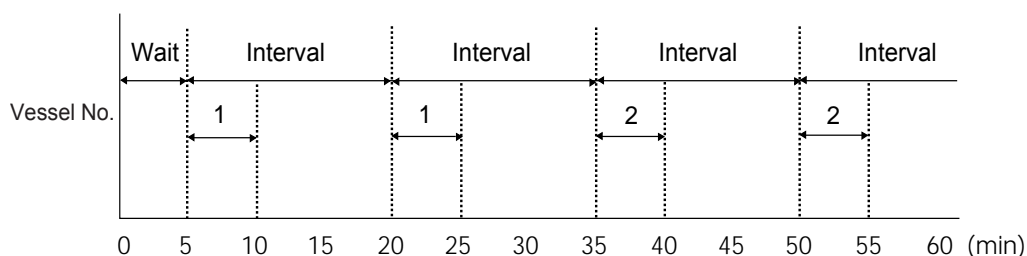
You can familiarize yourself with the SAMPLING mode by entering and running the sample program provided below. The table below shows the parameter values for the sample SAMPLING mode program.

**Figure 5–18: SAMPLING mode sample program parameters**

Parameter	Value
fraction	TIME
time.fraction	5 min
fractionating No.	2
wait.time	5 min
interval.time	15 min
repeat	2

When you run the sample program, the WFC III waits for 5 minutes and then begins the first 15-minute interval. Sample is collected into collection vessel number 1 for the first five minutes of the first interval. The second interval begins and sample is again collected into collection vessel number 1 for the first 5 minutes of the interval. In the third and fourth intervals, sample is collected into collection vessel number 2 in the same manner. The figure below shows the fraction collection pattern of the sample program.

**Figure 5–19: Collection pattern of the sample program**



## 5.5 Displaying the results of a run

The DISPLAY RESULT mode allows you to review the previous run as a table or a graph displayed on the LCD window. The table lists the events of the run along with the trigger, time, and duration of each event. The graph of the run, which shows signal input along with request and monitor times, is available only for runs processed in PEAK, WINDOW, and MANUAL modes.

**Tip:** Only the last run can be viewed in DISPLAY RESULT mode. Once you begin a new run, the results of the previous run are erased.

### 5.5.1 Table display

The table display can memorize 500 events from the previous run. In SAMPLING mode and all simple modes (TIME, DROP, SIGNAL, and VOLUME), if the number of events exceeds 500, only the first 500 events will be stored in memory. In PEAK and WINDOW modes, the zero event is overwritten when 500 events have been stored.

The table below lists the possible parameters displayed in a run result table.

**Table 5–11: Overview of DISPLAY RESULT tables**

Parameter		Description
V. No.	VESSEL NO.	0=drain, other collection vessels are numbered in collection order.



**Table 5–11: Overview of DISPLAY RESULT tables (continued)**

Parameter		Description
Event	START	Start of run, includes starting from NEXT.
	VALV.DRN	Draining at vessel position using a 3-way valve.
	PAUSE	Initiates pause.
	ADVANCE	Moves dropper assembly to the next collection vessel.
	END	Ends the run.
	TIME	Time collection or sampling time collection.
	DROP	Drop collection.
	SIGNAL	Signal collection.
	VOLUME	Volume or sampling volume collection.
	MONITOR	Monitor collection.
	NEXT	Waiting for the start signal in multi-sample mode.
	DRAIN	Draining at the drain position.
	REQUEST	Request collection.
Trigger	KEY	Event created by panel key.
	EXT	Event created by EXT. INPUT.
	RS232C	Event created using RS-232C.
	NORMAL	Event created by end conditions of normal collection mode.
Start time		Start time and elapsed time of an event. At NEXT, returns to 0.
Lap time		Duration period of an event.

The [figure "Sample run result table"](#) on page 106 shows a sample run result table.

**Figure 5–20: Sample run result table**

```

===== DISPLAY RESULT ===== (TIME
MODE)=====
V. No.  EVENT      TRIG      START T.      LAP TIME
-----
0        START      KEY              0m 30s
1        TIME              0m 30s      0m 20s
2        TIME              0m 50s      0m 20s
3        TIME              1m 10s      0m 20s
4        TIME              1m 30s      0m 20s
$

```

**To display run results as a table:**

1. Complete a run.
2. On the MAIN MENU, select **DISPLAY RESULTS** and press **ENTER**. A table of the run results appears.
3. To scroll through the pages, use the **UP** and **DOWN** keys.
4. To forward to the last event, hold down the **END** key and press **DOWN**.
5. To return to the first item, hold down the **END** key and press **UP**.
6. To return to the MAIN MENU, press **ESCAPE**.

**Tip:** When run results are displayed as a table, the lap time may not match the result time if the signal was manipulated during dropper assembly movement or if the wait time was not recognized.

## 5.5.2 Graph display

For runs processed in PEAK, WINDOW, and MANUAL modes, you can display the results of a run as a graph that shows signal input along with request and monitor times.

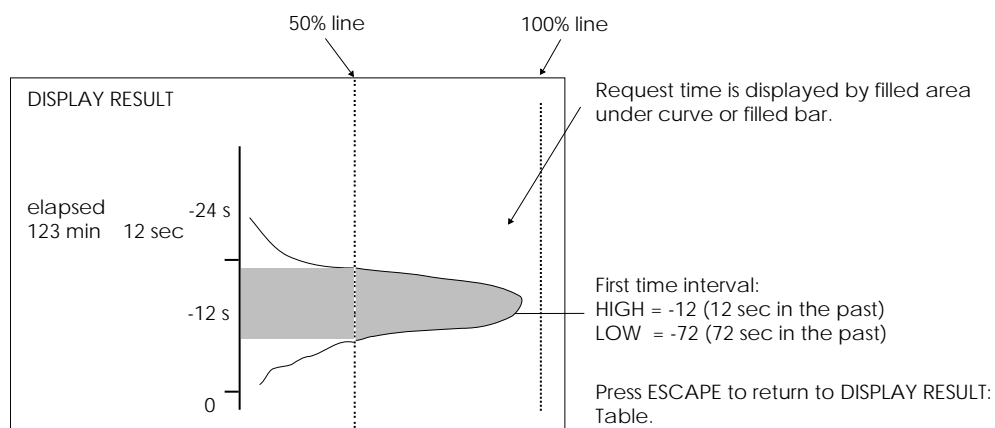
**To display run results as a graph:**

1. Complete a run.
2. On the MAIN MENU, select **DISPLAY RESULTS** and press **ENTER**. A table of the run results appears.
3. Press **ENTER** again to view the graph of the previous run. The display shows the first 30 seconds (at HIGH response rate) or 3 minutes (at low response rate) of the run.
4. Use the **UP** and **DOWN** keys to scroll through a total of 10 min (HIGH) or 60 min (LOW).

5. To forward to the last event, hold down the **END** key and press **DOWN**.
6. To return to the first item, hold down the **END** key and press **UP**.
7. To return to the run results table, press **ESCAPE**.

The figure below shows a sample graph display.

**Figure 5–21: Sample graph display**

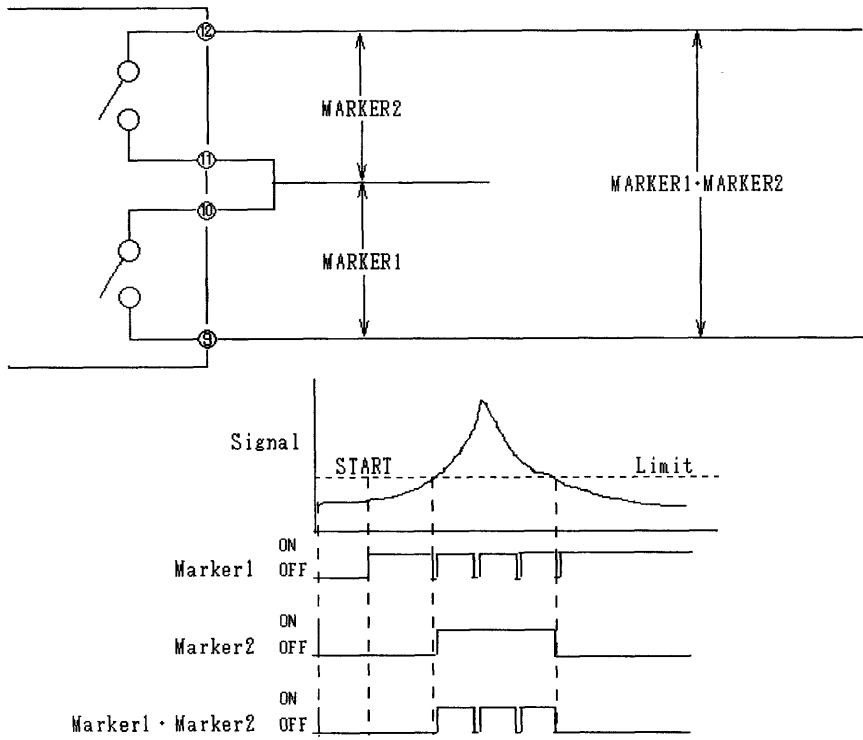


The WFC III can also display this type of graph while the run is in progress. Press **ENTER** during a run (after wait time) to view this display. See individual mode sections for more details.

## 5.6 Using event markers to track peaks

If you are using the WFC III in stand-alone mode, you can facilitate the location of peak fractions using the event marker signals. The event markers allow you to track the location of peak fractions within a rack. Event Marker 1 sends a tick signal each time the dropper moves to a new position for collection. Event Marker 2 is turned on at the start of collection and at the end of collection of the peak. The output from the two event markers are combined as shown in The figure below to delineate the number of positions used to collect each peak.

**Figure 5-22: Event marker I**



# 6 Maintenance

This chapter provides the maintenance guidelines and procedures necessary to maintain the instrument's performance.

Keep to a maintenance schedule, and perform maintenance as required and described in this chapter.

## 6.1 Spare parts

---

Refer to [Appendix C](#), for spare parts information. Parts not included in this appendix are not recommended for replacement by the customer.

### 6.1.1 Contacting Waters technical service

If you encounter any problems performing diagnostics or troubleshooting the WFC III, contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.) for assistance.

When you call Waters Technical Service, have the following information available:

- Completed Normal Operation Checklist for the method you are using
- Nature of the symptom
- WFC III serial number (located on the rear panel of the unit)
- Flow rate
- Operating pressure
- Mobile phase(s)
- Detector settings
- Type and serial number of column.

## 6.2 Safety and handling

---

When you troubleshoot the WFC III, keep the following safety considerations in mind:



**Warning:** To avoid possible electric shock,

- never disconnect an electrical assembly while power is applied to the WFC III. Once power is turned off, wait approximately 10 seconds before you disconnect an assembly.
- do not open the power supply cover. The power supply does not contain user-serviceable parts.



**Warning:** To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the WFC III. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.



**Notice:** To prevent circuit damage due to static charges, do not touch integrated circuit chips or other components that do not specifically require manual adjustment.

## 6.3 Running the test mode diagnostics

The test mode diagnostics allow you to verify that the WFC III is operating properly. The diagnostics are run from the TEST MODE menu. To access the TEST MODE menu, access the MAIN MENU, move the cursor to **[CONDITIONS]** and press **END**.

**Figure 6–1: TEST MODE menu**

=====TEST MODE=====	
NOZZLE MOVES :\$	[No. 1] UP or DOWN = moves\$
PANEL KEY :\$	[UP] press each key\$
3-WAY VALVE :\$	[ATTACH] UP = on valve change\$
I/O TERMINAL :\$	[I. START] 0. = output\$
CHROMATO SIG :\$	[0 - 10 mV] 2.0 mV\$
DROP LEVEL :\$	[ ] 0 drop LEVEL 0\$
[to MENU]\$	\$

### 6.3.1 Testing the dropper assembly movement

The dropper assembly delivers sample to the collection vessels. In order for the WFC III to operate correctly, the dropper assembly must move smoothly and stop in the correct positions.

**To test the dropper assembly movement:**

1. Install the standard test tube rack on the WFC III.

2. On the MAIN MENU, select [**CONDITIONS**] and press **END**. The TEST MODE menu appears.
3. Move the cursor to **NOZZLE MOVES : [No. 1]**. The dropper assembly moves to position number 1.
4. Check that the dropper assembly moves smoothly and stops directly over the collection vessel at the testing position.
5. Press **UP** to move the dropper assembly to the next test position.
6. Repeat steps 3 and 4 until the cycle is complete (the test position returns to No. 1).
7. Press **ESCAPE** to end the test.

You can perform the test in reverse order by following the procedure above and pressing **DOWN** instead of **UP**.

### 6.3.2 Testing the panel keys

The panel keys allow you to control the WFC III. You can use the panel key diagnostic test to determine if the keys are functioning properly.

#### To test the panel keys:

1. On the MAIN MENU, select [**CONDITIONS**] and press **END**. The TEST MODE menu appears.
2. Move the cursor to **PANEL KEY : [UP]** and press **UP**. The unit beeps to confirm the key is functioning correctly and PANEL KEY displays the next key to be pressed.
3. Continue pressing the keys displayed until the cycle is complete.

### 6.3.3 Testing the 3-way valve

The 3-way valve diverts the flow of solvent to a waste vessel as the dropper assembly moves between collection vessel positions. You can perform the 3-way valve test with or without the valve installed.

#### To test the 3-way valve:

1. On the MAIN MENU, select [**CONDITIONS**] and press **END**. The TEST MODE menu appears.
2. Move the cursor to **3-WAY VALVE : [ ]**. (ATTACH is displayed if a 3-way valve is installed. [NON] is displayed if the unit does not have a 3-way valve.) The dropper assembly moves to position number 119.
3. Press **UP** to turn the voltage on. The valve should open. If a 3-way valve is not installed, [OFF] will be displayed, and no voltage will be sent to the valve connector.
4. Press **ESCAPE** to end the test.

### 6.3.4 Testing the I/O terminal

The WFC III communicates with your HPLC system through the I/O terminal.

#### To test the I/O terminal:

1. On the MAIN MENU, select **[CONDITIONS]** and press **END**. The TEST MODE menu appears.
2. Move the cursor to **I/O TERMINAL : [I.START]** to check the external start input.
3. Create a short circuit between pins 3 and 4 to check the external start input. The unit beeps to confirm the input is functioning correctly.
4. Press **UP**. I/O TERMINAL displays I.END.
5. Check the external end input by creating a short circuit between pins 5 and 6. The unit beeps to confirm the input is functioning correctly.
6. Press **UP**. I/O TERMINAL displays I.COUNT.
7. Check the external count input by creating a short circuit between pins 7 and 8. The unit beeps to confirm the input is functioning correctly.
8. Press **UP**. I/O TERMINAL displays O.MARK1.
9. Check the event marker 1 output by testing pins 9 and 10 for an ON signal when you press **ENTER**.
10. Press **UP**. I/O TERMINAL displays O.MARK2.
11. Check the event marker 2 output by testing pins 11 and 12 for an ON signal when you press **ENTER**.

### 6.3.5 Testing the chromatography signal

#### To test the chromatography signal:

1. On the MAIN MENU, select **[CONDITIONS]** and press **END**. The TEST MODE menu appears.
2. Move the cursor to **CHROMATO SIG :[ ]**.
3. Use the **UP** and **DOWN** keys to select an input range (0 to 10 mV or 0 to 1 V). A readout of the chromatography signal is displayed to the right of [ ].

### 6.3.6 Testing the drop sensor

The WFC III uses a sensor light to count drops of sample. This allows the unit to collect fractions based on the number of drops of sample delivered to a collection vessel.

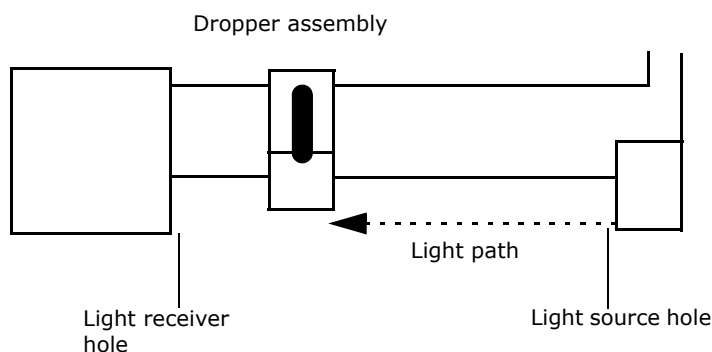
The sensor light is located in the carriage arm (see the [figure "Checking the drop sensor" on page 113](#)). The sensor light shines from the source hole (located on the right side of the carriage arm) to the receiver (located on the left side of the carriage arm).



### To test the drop sensor:

1. On the MAIN MENU, select **[CONDITIONS]** and press **END**. The TEST MODE menu appears.
2. Move the cursor to **DROP LEVEL :[ ]**. The dropper assembly moves to position 119 and the drop level (amount of light at the receiver) is displayed. Record the drop level value.
3. Cover the light source hole (located on the right side of the carriage arm). The unit beeps and the drop count increments.
4. Record the drop level value with the light source hole covered. If the difference between the initial drop level value (light source hole uncovered) and the current drop level value (light source hole covered) is more than 300, the drop sensor is operating correctly.

**Figure 6–2: Checking the drop sensor**



## 6.4 Troubleshooting

---

This section provides information for troubleshooting the Fraction Collector III.

**Tip:** Refer to ["Safety and handling" on page 109](#), for information on safety and handling considerations while you troubleshoot the Fraction Collector III.

### 6.4.1 Basic troubleshooting steps

Before you use the troubleshooting tables, follow these steps to isolate the cause of the hardware symptom.

#### To complete basic troubleshooting:

1. Step back and look at the system. Check the simple things first. Is something obvious causing the problem (for example, is an instrument unplugged or improperly connected)?
2. Compare current system operation with the way the system operated before the problem started. To help you identify normal operating conditions:
  - a. Record a map of your LC system (tubing and electrical connections).

- b. Keep a daily log.
- c. Run test chromatograms regularly.

It is important to keep track of system parameters and the results of your chromatography during normal operation. Troubleshooting is easier if you know the typical conditions when the system is operating correctly.

When your system is installed, and each time you develop a new method, fill out a Normal Operation Checklist to keep a record of system conditions during normal operation.

3. Identify in the following order the symptom that varies from normal system operation:
  - a. For each isolated symptom, identify a list of suspected causes. For suggested troubleshooting procedures, see the troubleshooting tables.
  - b. Run the performance tests for each chromatographic instrument to quickly determine if a problem exists with a particular instrument.
  - c. Refer to the troubleshooting information in the [table titled "General system troubleshooting" on page 114](#), the [table titled "Signal troubleshooting" on page 116](#), and the [table titled "Collection troubleshooting" on page 116](#). The troubleshooting tables allow you to narrow the possible cause of the symptom and find suggested corrective action for the cause you isolated.

If you determine that there is a problem related to another system component, refer to the appropriate operator's guide.

## 6.4.2 System troubleshooting

The table below provides troubleshooting suggestions for overall system problems that are not related to a particular module of the system.

**Table 6–1: General system troubleshooting**

Symptom	Possible cause	Corrective action
Unit does not power on	Power cord not connected	Check power cord.
	No power at outlet	Check line voltage.
	Power supply fuse blown	Call Waters Technical Service.
No response from keypad	Keypad is broken	Call Waters Technical Service.

**Table 6–1: General system troubleshooting (continued)**

Symptom	Possible cause	Corrective action
Screen is blank	Unit not powered on	Power on unit.
	Startup diagnostic test failure	Power off and on again. If failure not corrected, call Waters Technical Service.
	Display dim	Adjust LCD contrast using CONDITIONS mode.
	Display is broken	Call Waters Technical Service.
Front panel software "lock up"	Software problem or power line problem	Initialize unit's memory (see <a href="#">"Initializing the memory" on page 48</a> ). If failure not corrected, call Waters Technical Service.
LCD displays nonsense	Strong noise (static electric discharge)	Check for nearby noise source and remove.
LCD is completely dark or white	CPU was not reset	Power off unit, then power on unit. If failure not corrected, call Waters Technical Service.
	Strong noise (static electric discharge)	Check for nearby noise source and remove.
Displays power failure message without a power failure	Bad connection in AC plug	Check and replace.
	Power switch turned off during run	Turn on power switch.
Parameters not saved	Bad battery	Call Waters Technical Service.

### 6.4.3 Signal troubleshooting

The table below provides troubleshooting suggestions for problems related to signal input to the WFC III.

**Table 6-2: Signal troubleshooting**

Symptom	Possible cause	Corrective action
Cannot input chromatogram [SIGNAL]	Bad connecting cord	Check and replace.
	Bad I/O connector	Check and replace.
	Wrong connection with +/-	Check +/- connection and connect properly. The positive connection must be made to the even-numbered pin; the negative connection to the odd-numbered pin.
[EXT START] signal not accepted	Bad connecting cord	Check and replace.
	Bad I/O connector	Check and replace.
[EXT END] signal not accepted	Bad connecting cord	Check and replace.
	Bad I/O connector	Check and replace.
[EXT COUNT] signal not accepted	Bad connecting cord	Check and replace.
	Bad I/O connector	Check and replace.
Cannot input [EVENT MARKER] into a recorder	Bad connecting cord	Check and replace.
	Bad I/O connector	Check and replace.
[EVENT MARKER 2] signal not accepted	Bad connecting cord	Check and replace.
	Bad I/O connector	Check and replace.

### 6.4.4 Collection troubleshooting

The table below provides troubleshooting suggestions for problems encountered during collection runs.

**Table 6-3: Collection troubleshooting**

Symptom	Possible cause	Corrective action
Collection position out of alignment on X-axis (right/left)	Incorrect length of sample tubing	Check and connect properly.
	X-slider mechanism may be corroded or dusty	Clean and lubricate with silicone oil.
Collection position out of alignment on Y-axis (front/back)	Incorrect length of Teflon tubing	Check and connect properly.
	Y-slider mechanism may be corroded or dusty	Clean and lubricate with silicone oil.

**Table 6–3: Collection troubleshooting (continued)**

<b>Symptom</b>	<b>Possible cause</b>	<b>Corrective action</b>
Collection volume varies in DROP mode	Other light sources interfere with drop sensor	Check to see if light from windows or other sources shines on sensor. Remove or block other light sources or relocate WFC III.
	Vibration interferes with proper collection	Check for compressor or other nearby source of vibration. Remove source of vibration or relocate WFC III.
	Bubbles in buffer	Check sealing at joint for completeness. Reseal if necessary.
	Crystals stuck to end of tubing at dropper assembly	Remove crystals stuck to tubing and flush tubing with clean solvent.
	Tubing end at dropper assembly not cut straight	Recut tubing at a 90° angle.
	Test tubes and dropper assembly not centered	Check the positioning and readjust to center.
	Tubing at dropper assembly too short	Adjust tubing so that 3 to 5 mm protrudes from dropper assembly.
No collection in DROP mode	Protruding length of tubing at dropper assembly is longer than 5 mm	Adjust tubing so that 3 to 5 mm protrudes from dropper assembly.
	Other light sources interfere with drop sensor	Check to see if light from windows or other sources shines on sensor. Remove or block other light sources or relocate WFC III.
	Teflon tubing interferes with light pass	Check and adjust tubing.

**Table 6-3: Collection troubleshooting (continued)**

<b>Symptom</b>	<b>Possible cause</b>	<b>Corrective action</b>
Collection times vary in TIME and VOLUME modes	Bubbles in buffer	Check sealing at joint for completeness. Reseal if necessary.
	Crystals stuck to end of tubing at dropper assembly	Remove crystals stuck to tubing and flush tubing with clean solvent.
	Tubing end at dropper assembly not cut straight	Recut tubing at a 90° angle.
	Improper positioning of both center of tubes and dropper assembly	Check tube rack position and reinstall properly.
Incorrect count time in TIME mode	Extreme variation in operating temperatures	Control ambient temperature.
	Software problem	Check for reproducibility of error, replace ROM if bad.
Cannot collect in PEAK mode both or either absolute and slope values	Bad connecting cords between detector and WFC III	Check and replace.
	Bad I/O connector	Check and replace.
	Connection polarity +/- is incorrect	Check +/- connection and connect properly. The positive connection must be made to the even-numbered pin; the negative connection to the odd-numbered pin.
	Collection parameters set incorrectly	Check and reset.

# A Safety Advisories


Waters instruments and devices display hazard symbols that alert you to the hidden dangers associated with a product's operation and maintenance. The symbols also appear in product manuals where they accompany statements describing the hazards and advising how to avoid them. This appendix presents the safety symbols and statements that apply to all of Waters' product offerings.


## A.1 Warning symbols


---

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with the misuse of an instrument or device. Heed all warnings when you install, repair, or operate any Waters instrument or device. Waters accepts no liability in cases of injury or property damage resulting from the failure of individuals to comply with any safety precaution when installing, repairing, or operating any of its instruments or devices.


The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device or component of an instrument or device. When one of these symbols appear in a manual's narrative sections or procedures, an accompanying statement identifies the applicable risk and explains how to avoid it.


 **Warning:** (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)


 **Warning:** (Risk of burn injury from contacting hot surfaces.)


 **Warning:** (Risk of electric shock.)

 **Warning:** (Risk of fire.)

 **Warning:** (Risk of sharp-point puncture injury.)

 **Warning:** (Risk of hand crush injury.)

 **Warning:** (Risk of injury caused by moving machinery.)

 **Warning:** (Risk of exposure to ultraviolet radiation.)

 **Warning:** (Risk of contacting corrosive substances.)



**Warning:** (Risk of exposure to a toxic substance.)



**Warning:** (Risk of personal exposure to laser radiation.)



**Warning:** (Risk of exposure to biological agents that can pose a serious health threat.)



**Warning:** (Risk of tipping.)



**Warning:** (Risk of explosion.)



**Warning:** (Risk of high-pressure gas release.)

## A.1.1 Specific warnings

The following warnings (both symbols and text) can appear in the user manuals of particular instruments and devices and on labels affixed to them or their component parts.

### A.1.1.1 Burst warning

This warning applies to Waters instruments and devices fitted with nonmetallic tubing.



**Warning:** To avoid injury from bursting, nonmetallic tubing, heed these precautions when working in the vicinity of such tubing when it is pressurized:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to compounds with which it is chemically incompatible: tetrahydrofuran, nitric acid, and sulfuric acid, for example.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, significantly reducing the pressure at which the tubing can rupture.



### A.1.1.2 Biohazard warning

The following warning applies to Waters instruments and devices that can process material containing biohazards, which are substances that contain biological agents capable of producing harmful effects in humans.



**Warning:** To avoid infection with potentially infectious, human-sourced products, inactivated microorganisms, and other biological materials, assume that all biological fluids that you handle are infectious.

Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the biohazard safety representative for your organization regarding the proper use and handling of infectious substances.

### A.1.1.3 Biohazard and chemical hazard warning

These warnings apply to Waters instruments and devices that can process biohazards, corrosive materials, or toxic materials.



**Warning:** To avoid personal contamination with biohazards, toxic materials, or corrosive materials, you must understand the hazards associated with their handling. Guidelines prescribing the proper use and handling of such materials appear in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards*.

Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the safety representative for your organization regarding its protocols for handling such materials.

## A.2 Notices

---

Notice advisories appear where an instrument or device can be subject to use or misuse that can damage it or compromise a non-clinical sample's integrity. The exclamation point symbol and its associated statement alert you to such risk.

**!** **Notice:** To avoid damaging the instrument's case, do not clean it with abrasives or solvents.

## A.3 Bottles Prohibited symbol

---

The Bottles Prohibited symbol alerts you to the risk of equipment damage caused by solvent spills.



**Prohibited:** To avoid equipment damage caused by spilled solvent, do not place reservoir bottles directly atop an instrument or device or on its front ledge. Instead, place the bottles in the bottle tray, which serves as secondary containment in the event of spills.

## A.4 Required protection

---

The Use Eye Protection and Wear Protective Gloves symbols alert you to the requirement for personal protective equipment. Select appropriate protective equipment according to your organization's standard operating procedures.



**Requirement:** Use eye protection when refilling or replacing solvent bottles.





**Requirement:** Wear clean, chemical-resistant, powder-free gloves when handling samples.


## A.5 Warnings that apply to all Waters instruments and devices


---


When operating this device, follow standard quality-control procedures and the equipment guidelines in this section.


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


 **Important:** Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.


 **Achtung:** Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.


 **Avvertenza:** qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.


 **Atencion:** cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

 **注意:** 未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

 **注意:** 未經有關法規認證部門明確允許對本設備進行的改變或改裝,可能會使使用者喪失操作該設備的合法性。

 **주의:** 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

 **注意:** 規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

 **Warning:** Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.



**Attention:** Manipulez les tubes en polymère sous pression avec précaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Éteignez toute flamme se trouvant à proximité de l'instrument.
- Évitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Évitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.



**Vorsicht:** Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.

Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.



**Attenzione:** fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.



**Advertencia:** se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.

Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.



**警告：**當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癢或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。

要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。



**警告：**当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。

要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。



**경고:** 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.

염화 메틸렌 (Methylene chloride) 및 디메틸술폭시드 (Dimethyl sulfoxide) 는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오 .



**警告：**圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。

塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があります、その場合、チューブは極めて低い圧力で破裂します。



**Warning:** The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.



**Attention:** L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.



**Vorsicht:** Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.



**Attenzione:** si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.



**Advertencia:** el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.



**警告：**使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。



**警告：**使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。



**경고：**제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.



**警告：**ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

## A.6 Warnings that address the replacing of fuses

The following warnings pertain to instruments and devices equipped with user-replaceable fuses. Information describing fuse types and ratings sometimes, but not always, appears on the instrument or device.

### Finding fuse types and ratings when that information appears on the instrument or device



**Warning:** To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.



**Attention:** pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boîte à fusible de l'instrument.



**Vorsicht:** Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.



**Attenzione:** per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.



**Advertencia:** Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.



**警告：**為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。



**警告：**为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。



**경고：**화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.



**警告：**火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

## Finding fuse types and ratings when that information does not appear on the instrument or device



**Warning:** To protect against fire, replace fuses with those of the type and rating indicated in the "Replacing fuses" section of the Maintenance Procedures chapter.



**Attention:** pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués dans la rubrique "Remplacement des fusibles" du chapitre traitant des procédures de maintenance.



**Vorsicht:** Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert im Abschnitt "Sicherungen ersetzen" des Kapitels "Wartungsverfahren" angegeben sind.



**Attenzione:** per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate nel paragrafo "Sostituzione dei fusibili" del capitolo "Procedure di manutenzione".



**Advertencia:** Para evitar incendios, sustituir los fusibles por aquellos del tipo y características indicados en la sección "Sustituir fusibles".



**警告：**為了避免火災，更換保險絲時，應使用「維護步驟」章節中「更換保險絲」所指定之相同類型與規格的保險絲。



**警告：**為了避免火災，應更換“維護步驟”一章的“更換保險絲”一節中介绍的相同类型和规格的保险丝。



**경고：**화재의 위험을 막으려면 유지관리 절차 단원의 "퓨즈 교체" 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.















**警告：**火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

## A.7 Electrical and handling symbols

---

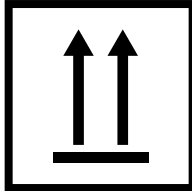

### A.7.1 Electrical symbols

The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument's front or rear panels.


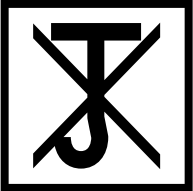
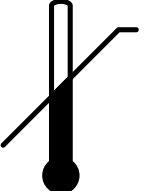
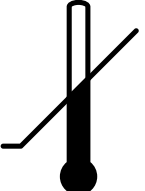
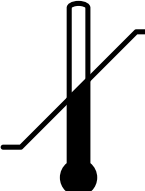
Symbol	Description
	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Alternating current (3 phase)
	Safety ground
	Frame, or chassis, terminal
	Fuse
	Functional ground
	Input
	Output

## A.7.2 Handling symbols

The following handling symbols and their associated statements can appear on labels affixed to the packaging in which instruments, devices, and component parts are shipped.

Symbol	Description
	Keep upright!
	Keep dry!



Symbol	Description
	Fragile!
	Use no hooks!
	Upper limit of temperature
	Lower limit of temperature
	Temperature limitation



# B Specifications

This appendix includes specifications for the following:

- Physical
- Environmental
- Electrical
- 3-way valve
- External component materials

**Table B-1: Physical specifications**

<b>Item</b>	<b>Specification</b>
Height	13 1/4 inches (335 mm)
Depth	15 1/4 inches (387 mm)
Width	11 1/4 inches (286 mm)
Weight	15.4 lbs. approximate (7 kg approximate)

**Table B-2: Environmental specifications**

<b>Item</b>	<b>Specifications</b>
Installation site	Indoor use (pollution degree 1 or 2 in accordance with IEC664)
Altitude	6562 feet (2000 m)
Over voltage category	II (local level)
Operating temperature	4 to 40 °C (39 to 104 °F)
Operating humidity	20 to 85%, noncondensing
Storage humidity	20 to 90%, noncondensing
Solvent compatibility	Solvents consistent with materials of construction. See the <a href="#">table titled "Materials of external components" on page 132</a> and <a href="#">Appendix D</a> .

**Table B-3: Electrical specifications**

Item	Specification
Power requirements	Grounded ac outlet 100/240 Vac, main voltage fluctuations not to exceed +/- 10% of normal voltage CSA certification is approved for 100 V, 115 V, and 240 V.
Frequency	50 to 60 Hz, single phase
Fuse	250 V, 3 A time delay
Current rating	0.23 A at 100 V, 0.2 A at 115 V, 0.15 A at 240 V

**Table B-4: 3-way valve specifications**

Item	Standard valve	Low residual valve
Valve orifice	1.6-mm diameter	1.2-mm diameter
Maximum pressure	IN = 294 Kpa (43 psi) OUT = 98 Kpa (14 psi) (NC/NO)	IN = 196 Kpa (28 psi) OUT = 49 Kpa (7 psi) (NC/NO)
Residual volume	IN = 26 $\mu$ L OUT = 66 $\mu$ L (NC)/ 66 $\mu$ L (NO)	IN = 14 $\mu$ L OUT = 7 $\mu$ L (NC)/ 7 $\mu$ L (NO)
Coefficient of Volumetric Flow ( $C_v$ )	0.065	0.025
Solvent temperature	0 to 60 °C	0 to 60 °C
Power consumption	2.2 VA maximum	2.2 VA maximum
External dimensions	25 mm diameter $\times$ 51 mm length	25 mm diameter $\times$ 51 mm length
Weight	96 g	96 g

**Table B-5: Materials of external components**

Component	Material
Front and top housing	ABS
Back housing	Aluminum
Drain, rack, and drip tray	Polypropylene
Tubing collar	Nylon (Polyamide)
Dust cover	PVC
Panel sheet	PET
Drain tube	Silicone rubber
Dropper assembly	Stainless steel
Mounting plate	Stainless steel

# C Spare Parts and Accessories

This appendix includes information on recommended spare parts and accessories for the Waters Fraction Collector III.

## C.1 Fraction Collector III spare parts

---

The table below lists the spare parts available for the WFC III. Parts not listed in the table are not recommended for customer replacement.

**Table C-1: Recommended spare parts for the Fraction Collector III**

Item	Part number
Dropper Holder	700000311
Dust Cover	700000144
Drain	700000314
Knob, Height Adjustment	700000315
WFC I/O Connector	700000328
Right Arm Cover	700000156
Left Arm Cover	700000153
Front Cover	700002163
Front, Drip Tray	700002164

## C.2 Fraction Collector III accessories

---

The table below lists the accessories that are available for the Fraction Collector III.

**Table C-2: Fraction Collector III accessories**

Item	Part number
3-way valve (standard)	725000142
3-way valve (low-residual)	725000143
Extension nozzle	725000151
High-flow dropper assembly	WAT037097
Drip tray	700002164

**Table C-2: Fraction Collector III accessories (continued)**

<b>Item</b>	<b>Part number</b>
LC Connection Kit (supplied with the WFC III)	WAT037048
Tygon tubing, 6.35 mm ID × 9.52 mm OD × 5 m length, pack of 2 (for use with prep funnel rack)	WAT037047
Teflon tubing 8 mm ID × 50 feet length (for use with prep funnel rack)	WAT037090
Standard test tube rack (supplied with the WFC III)	725000152
Tube supports for 12-mm OD tubes, pack of 30	725000153
Internal support plate for use with short length tubes in standard test tube rack	725000154
17-mm OD vial rack	725000146
28-mm OD vial rack	725000147
Carousel rack	725000144
Eppendorf tube rack	725000145
Micro-titer plate rack	725000110
Multi-purpose rack	725000113
Multi-purpose rack adapter	725000120
Prep funnel rack	725000148
Prep funnel	725000149
Tabletop rack (for use with prep funnel rack)	289000440
Front cover	700002163
Left side cover	700000153
Right side cover	700000156
<i>Waters Fraction Collector III Operator's Guide</i> (supplied with the WFC III)	71500012102

# D Chemical Compatibility

This appendix provides information on the compatibility of the Waters Fraction Collector III's components with a variety of chemicals.

## D.1 Introduction

---

### D.1.1 Clean solvents

Clean solvents provide:

- Reproducible results
- Operation with minimal instrument maintenance

A dirty solvent can cause:

- Baseline noise and drift
- Blockage of the solvent reservoir and inlet filters with filtered particulate matter

### D.1.2 Solvent quality

Use LC/MS-grade solvents to ensure the best possible results. Filter through 0.45- $\mu\text{m}$  filters before use. Solvents distilled in glass generally maintain their purity from lot to lot; use them to ensure the best possible results.

### D.1.3 Preparation checklist

The following solvent preparation guidelines help to ensure stable baselines and good resolution:

- Filter solvents with a 0.45- $\mu\text{m}$  filter.
- Degas and/or sparge the solvent.
- Stir the solvent.
- Keep in a place free from drafts and shock.

### D.1.4 Water

When using water, use a high quality source such as a Milli-Q<sup>®</sup> water purification system. If the water system does not provide filtered water, filter it through a 0.45- $\mu\text{m}$  membrane filter before use.

## D.1.5 Buffers

When using buffers, dissolve salts first, adjust the pH, and then filter to remove insoluble material.

## D.1.6 THF

When using unstabilized THF, ensure that your solvent is fresh. Previously opened bottles of THF contain peroxide contaminants, which cause baseline drift.



**Warning:** THF contaminants (peroxides) are potentially explosive if concentrated or taken to dryness.

## D.2 Component chemical compatibility

The table below lists the chemical compatibility with the various component materials of the Waters Fraction Collector III.

**Table D-1: Component chemical compatibility**

Chemical	Polypropylene	ABS	Polyamide	Aluminum	Stainless steel	Teflon (PTFE)	Diflon (PCTFE)
Acetaldehyde	R	X	—	—	—	R	—
Acetic acid (10%)	R	P	X	P	P	R	R
Acetic acid, Glacial	R	X	—	P	X	R	R
Acetone	R	X	—	R	R	R	R
Acetone (10%)	P	P	P	R	R	R	R
Acetonitrile	P	—	—	—	R	R	R
Aluminum sulfate	R	R	X	P	R	R	—
Ammonia, liquid	R	—	—	R	R	R	R
Ammonium acetate	R	—	—	R	—	R	R
Ammonium carbonate	R	R	—	P	R	R	R
Ammonium chloride	R	R	R	P	P	R	R
Ammonium hydroxide	R	P	R	P	R	R	R
Ammonium nitrate	R	—	X	—	R	R	R
Ammonium sulfate	R	R	—	R	R	R	R

**R=Recommended P=Possible X=Not recommended —=Insufficient data**



**Table D-1: Component chemical compatibility (continued)**

<b>Chemical</b>	<b>Polypropylene</b>	<b>ABS</b>	<b>Polyamide</b>	<b>Aluminum</b>	<b>Stainless steel</b>	<b>Teflon (PTFE)</b>	<b>Diflon (PCTFE)</b>
Amyl acetate	P	X	—	R	R	R	R
Amyl alcohol	R	R	R	P	R	R	—
Aniline	P	X	—	P	R	R	R
Benzaldehyde	X	P	—	P	P	R	—
Benzene	X	X	—	P	R	R	R
Benzenesulfonic acid	X	—	—	X	P	R	—
Benzoyl chloride	X	X	R	X	P	—	—
Benzyl alcohol	—	X	P	P	R	—	—
Boric acid	R	—	—	X	P	R	—
Butane	P	R	R	P	R	R	—
Butanol	R	—	—	P	R	R	—
Butyl acetate	P	—	—	R	R	R	R
Butyl Phthalate	P	—	R	P	P	R	—
Butyric acid	P	X	—	P	R	R	—
Calcium bisulfate	R	P	—	—	—	—	R
Calcium chloride	R	R	R	X	P	R	R
Calcium hydroxide	R	—	P	P	P	R	R
Calcium nitrate	R	R	X	P	P	R	R
Carbon disulfide	X	—	—	R	R	R	
Carbon tetrachloride	P	X	R	X	R	R	P
Chlorobenzene	P	X	—	R	R	P	R
Chloroform	P	X	—	P	R	P	R
Ethanol	R	R	—	P	R	R	R
Ethyl acetate	P	X	—	R	P	R	R
Hexane	P	X	—	R	R	R	R
Hydrochloric acid, 6N	P	R	—	X	X	R	R
Hydrogen peroxide (10%)	R	R	—	R	R	R	—
Isopropyl alcohol	R	—	—	P	P	R	—
Methanol	R	X	—	P	R	R	R
Sodium hydroxide	R	P	—	X	P	R	R
<b>R=Recommended P=Possible X=Not recommended —=Insufficient data</b>							

**Table D-1: Component chemical compatibility (continued)**

<b>Chemical</b>	<b>Polypropylene</b>	<b>ABS</b>	<b>Polyamide</b>	<b>Aluminum</b>	<b>Stainless steel</b>	<b>Teflon (PTFE)</b>	<b>Diflon (PCTFE)</b>
Sodium hypochlorite (<20%)	R	P	—	X	P	R	R
Sulfuric acid, 6N	R	P	—	X	X	R	R
Tetrahydrofuran	P	—	—	—	R	R	P
<b>R=Recommended P=Possible X=Not recommended —=Insufficient data</b>							