

# **Waters 2757 Sample Manager, Collector Installation and Maintenance Guide**

71500275704/Revision B

# Waters

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

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| Conventional mail | Waters Corporation<br>34 Maple Street<br>Milford, MA 01757<br>USA   |

## Safety considerations

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Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. You must know the potentially hazardous effects of all substances you work with. Always follow

Good Laboratory Practice, and consult your organization's safety representative for guidance.

## Considerations specific to the 2757 Sample Manager

**Tip:** When you use the instrument, follow generally accepted procedures for quality control and methods development.

If you observe a change in the retention of a particular compound, in the resolution between two compounds, or in peak shape, immediately determine the reason for the changes. Until you determine the cause of a change, do not rely on the separation results.

**Tip:** The Installation Category (Overvoltage Category) for this instrument is Level II. The Level II Category pertains to equipment that receives its electrical power from a local level, such as an electrical wall outlet.

## Safety advisories

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

## Operating this instrument

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

## Applicable symbols

| Symbol   | Definition   |
|--|--|
|  Waters Corporation<br>34 Maple Street Milford, MA 01757 U.S.A.                           | Manufacturer location  |
|  Waters Corporation<br>Floats Road<br>Wythenshawe<br>Manchester M23 9LZ<br>United Kingdom | Authorized representative of the European Community  |
|   | Confirms that a manufactured product complies with all applicable European Community directives                  |
|  ABN 49 005 444 751   | Australia C-Tick EMC Compliant   |
|   | Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements |
|   | Consult instructions for use   |

## Audience and purpose

This guide is intended for personnel who install and maintain the Waters 2757 Sample Manager.

## Intended use of the Waters 2757 Sample Manager

The Waters 2757 Sample Manager is for research use only.

## Calibrating

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

When calibrating mass spectrometers, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide, not operator's guide, accompanies the

instrument, consult the instrument's online Help system for calibration instructions.

## Quality-control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. 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.

## ISM classification

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### ISM Classification: ISM Group 1 Class B

This classification has been assigned in accordance with 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

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# 1 Introduction

This chapter describes the Waters® 2757 Sample Manager, which is a high-capacity sample processing system for collecting purified fractions from either a:

- High-performance liquid chromatography (HPLC) system
- Liquid chromatography/mass spectrometry (LC/MS) system

The 2757 Sample Manager automates collecting fractions into deep-well microplates (MTP), test tubes, scintillation vials, or conventional autosampler vials.

The 2757 Sample Manager connects to the computer, running MassLynx™ software version 3.5x or higher, through an RS-232 port.

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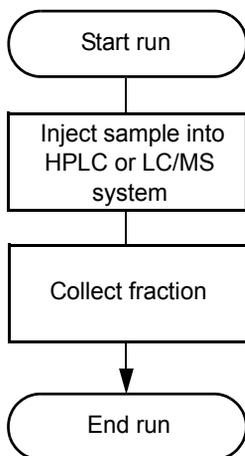
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## Sample flow

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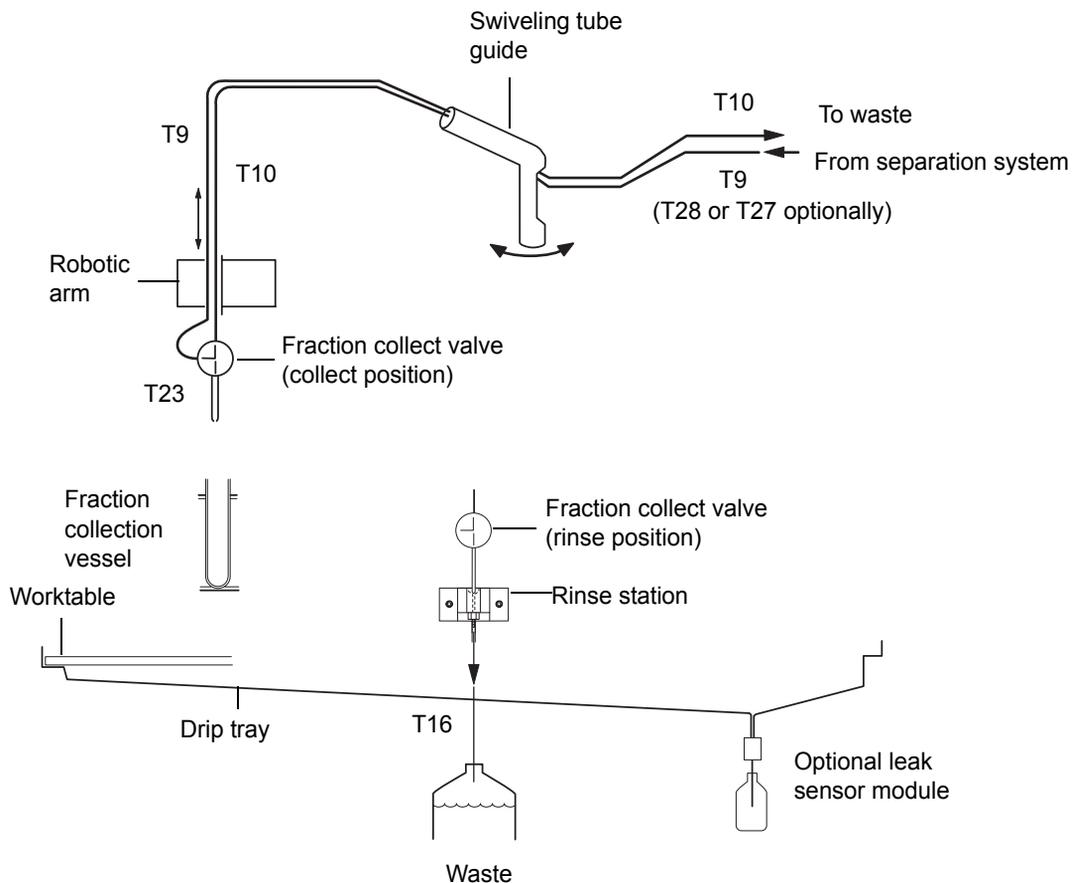
During operation, the sample flows through the HPLC or LC/MS system into the 2757 Sample Manager for fraction collection.

### Typical sample flow



The figure below shows the solvent flow path through the 2757 Sample Manager.

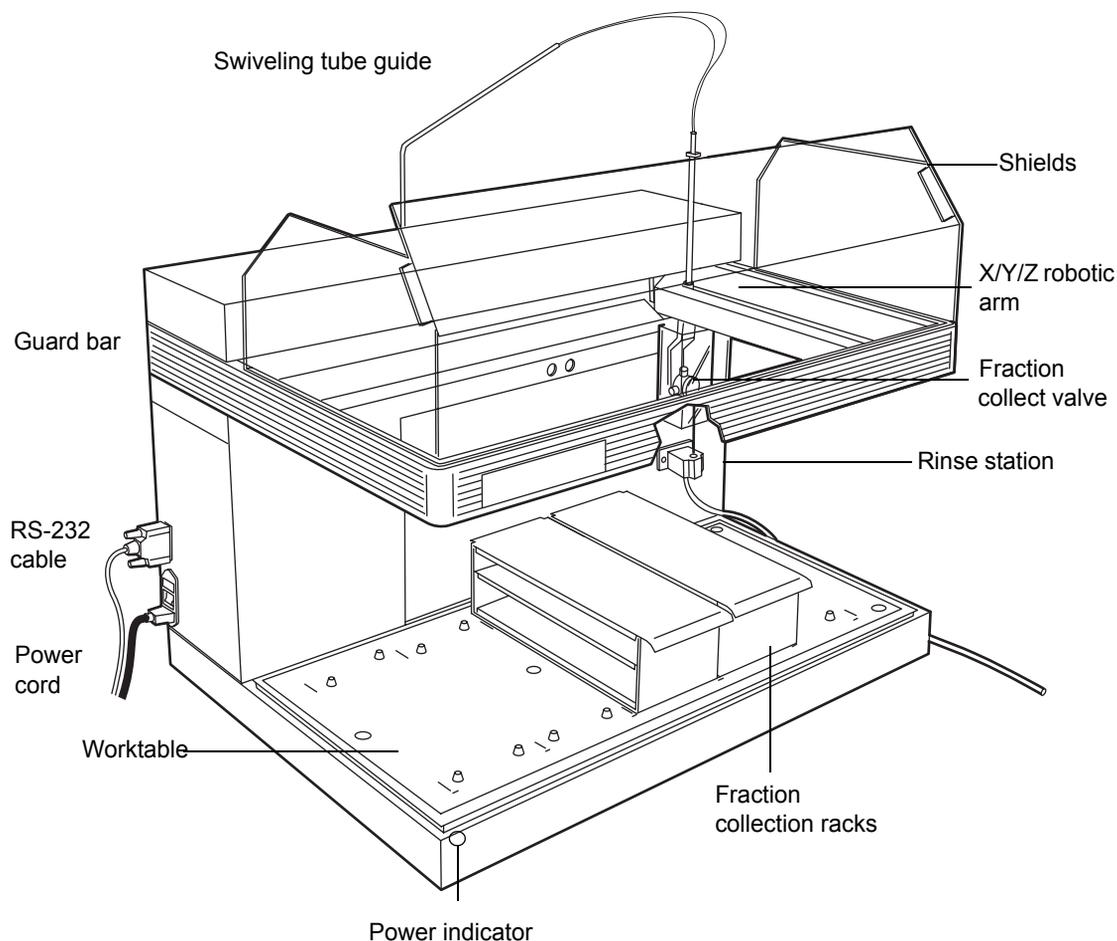
## 2757 Sample Manager fluidic path



## Hardware

The major components of the 2757 Sample Manager hardware are shown in the figure below.

## Major hardware components



## Worktable

The worktable is the space under the fraction dispense tube and on top of the drip tray that holds the fraction collection racks. To place the racks in the correct location, match the actual rack on the worktable to the type and location shown on the Inlet Editor window in the MassLynx software. Align each rack with the marks on the worktable and slide the rack toward the back of the worktable to engage the locator pins. A drip tray underneath the worktable catches spills and drains into a dedicated waste container.

## Fraction collection racks

The following racks are used for fraction collection. The test tubes or vials must not be covered as the fraction dispense tube will not penetrate a cover.

- **13-mm tubes** – 112-test tube rack for 13 × 100 mm test tubes
- **16-mm tubes** – 84-test tube rack for 16 × 100 to 150 mm test tubes
- **18-mm tubes** – 78-test tube rack for 18 × 150 mm test tubes
- **25-mm tubes** – 36-test tube rack for 25 × 150 mm test tubes
- **28-mm scintillation vials** – 32-vial rack for 28-mm diameter scintillation vials
- **MTP deep** – Racks hold three 96-well deep format microtiter plates



**Caution:** Test tubes less than 100 mm tall are not supported.

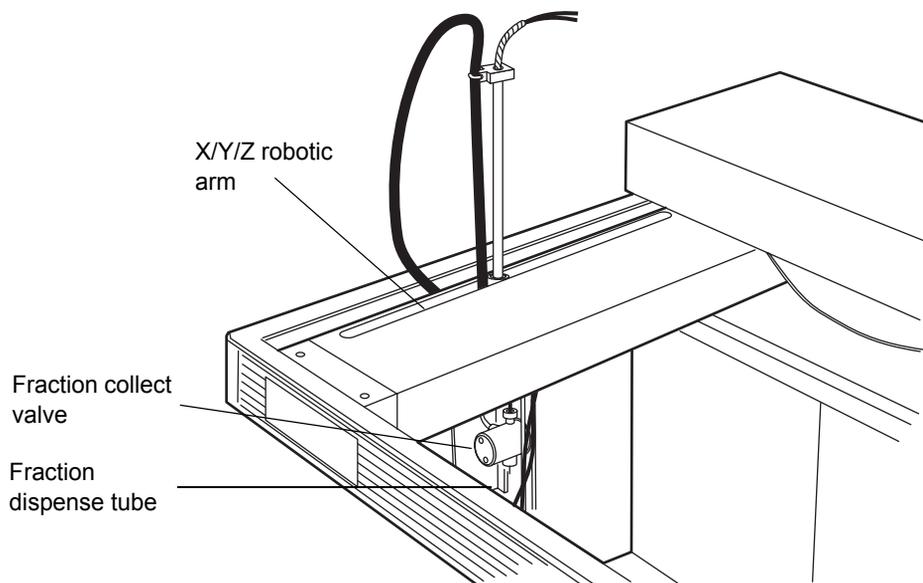
## X/Y/Z robotic arm

The Waters 2757 Sample Manager contains a robotic module that moves the fraction dispense tube in three axes:

- The x-axis moves the fraction dispense tube to the left and right.
- The y-axis moves the fraction dispense tube forward and backward.
- The z-axis moves the fraction dispense tube up and down.

The major components of the robotic module are in the [figure “X/Y/Z robotic arm” on page 1-6](#). Sample coming from the detector(s) is routed to the fraction collect valve and fraction dispense tube which delivers it to the collection tubes, vials, or deep wells.

## X/Y/Z robotic arm



## Wash station

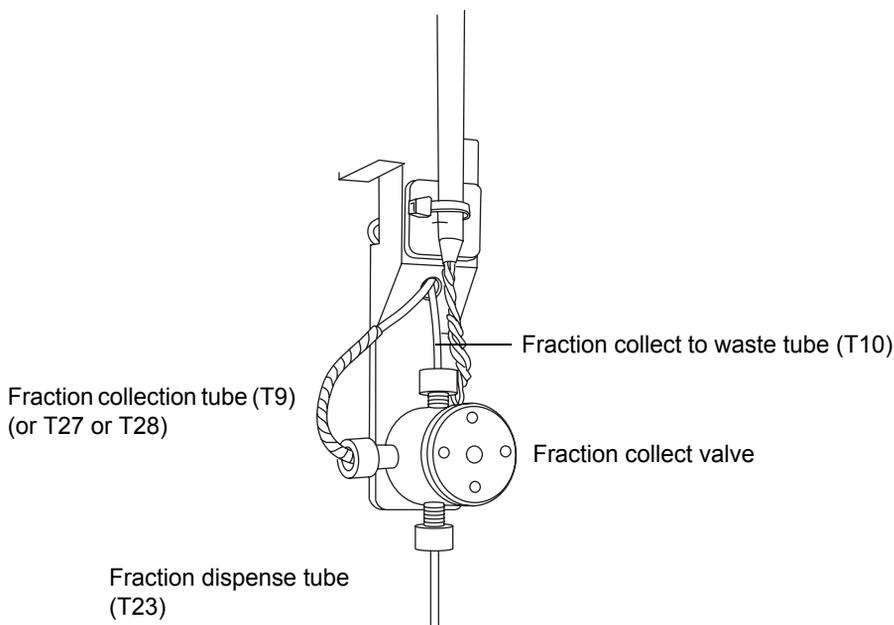
The wash station is the default position for rinsing the fraction collection tube. Solvent flows from the detector or separation system through the fraction collect valve and fraction dispense tube into the wash station. The wash station drains into a waste container (not provided) under the 2757 Sample Manager.

## Fraction collection mechanism

The fraction collection mechanism consists of the fraction collection tube, fraction collect valve, and fraction dispense tube. The fraction collection tube connects the UV detector outlet or flow splitter depending on system configuration to the fraction dispense valve. The standard 2757 Sample Manager is equipped with a 10-foot long fraction collection tube (T9) with a 0.020-inch ID. For best performance at higher and lower flow rates, optional fraction collection tubes are available:

- T27 for low flow rates, 0.010-inch ID
- T28 for high flow rates, 0.040-inch ID

## Fraction collection mechanism



## Spare parts and options

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You can customize the 2757 Sample Manager with the following items to suit your application and site requirements (see [Appendix C, Spare Parts and Options](#)).

### Racks

The 2757 Sample Manager uses racks to hold test tubes, scintillation vials, or microplates. The instrument is not provided with racks, sample containers, solvent containers, or waste fluid containers.

**Tip:** At least one fraction collection rack is necessary for system operation.

### Fume hood

You can equip the 2757 Sample Manager with a fume hood (part number 205000125) to minimize solvent vapors that may escape into the laboratory air when collecting sample fractions.

## Leak sensor module

Waters strongly recommends that you install a leak sensor module to monitor the flow from the drip tray drain tube into the waste container. If flow is measured, the Waters leak sensor module shuts off the pump(s) via software control to prevent solvent overflow and a warning message appears. The leak sensor module has an auxiliary signal which can be used to trigger a user-supplied shut-off valve for high-capacity solvent containers.



**Caution:** Do not route the wash station drain tube into the leak sensor module. If the wash station drain tube is incorrectly routed to the leak sensor module, the normal flow of wash solvent will shut off the pump.

# 2 Unpacking and Installing the 2757 Sample Manager

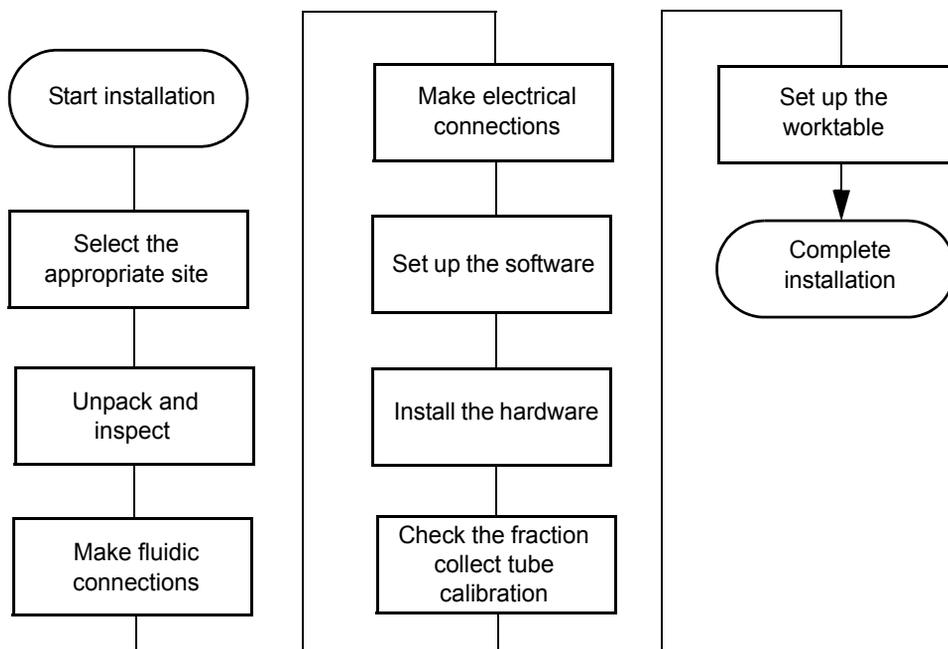
This chapter describes how to install the Waters 2757 Sample Manager. The figure below shows the primary installation steps.

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**Tip:** To install the 2757 Sample Manager, you should know how to set up and operate general laboratory instruments and computer-controlled devices, and how to handle solvents.

### Primary installation steps



## Site selection and power requirements

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### Required materials

To install the instrument, you need the Startup Kit (see [Appendix C, Spare Parts and Options](#)), which includes tools such as a flat-blade screwdriver and a metric Allen wrench set. You also need:

- Racks (not included) for fraction collection
- Fraction containers such as 2-mL vials, 13 × 100 mm test tubes or microplates
- Waste fluid container large enough for system use

### Site selection

Select a site for the 2757 Sample Manager that meets the requirements in the table below.

#### Installation site requirements

| Factor             | Requirements  |
|--------------------|---|
| Temperature        | 15 to 40 °C (59 to 104 °F)  |
| Relative humidity  | Maximum relative humidity 85% for temperatures up to 31 °C, decreasing linearly to 50% relative humidity at 40 °C   |
| Bench space        | Width: 34 in. (86.4 cm)<br>Depth: 24.5 in. (62.2 cm)<br>Height: 30 in. (76.2 cm)<br>Height: 33 in. (83.8 cm) with optional fume hood<br>Level to within ±2° |
| Weight             | 120 lbs. (59.4 kg)  |
| Vibration          | Negligible  |
| Clearance          | At least 1 in. (2.5 cm) at the back for ventilation.<br>Space for fluid waste container below the instrument.   |
| Static electricity | Negligible  |
| Power              | Autoselecting 110/230 Vac ±10%,<br>47 to 63 Hz, maximum 300 watts   |

## Installation site requirements (Continued)

| Factor                 | Requirements   |
|------------------------|--|
| Electromagnetic fields | Negligible. No nearby source of electromagnetic noise, such as arcing relays or electric motors. |
| Fume hood (optional)   | 4-in. duct with greater than 80 CFM flow   |

## Power requirements

The 2757 Sample Manager requires the following:

- Grounded alternating current (ac) power source
- Minimal power transients and fluctuations



**Warning:** To avoid possible electric shock, always turn off the instrument and unplug the power cord before performing maintenance procedures.



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

## Inspecting and unpacking

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The components of the 2757 Sample Manager are packed and shipped in one or more cartons that contain the following items:

- Waters 2757 Sample Manager
- Certificate of Validation
- Power cord, signal cables, and shields
- Release notes
- Startup Kit
- Optional fume hood (separate carton)
- Optional racks (separate carton)

## Inspecting the shipping container

Inspect the exterior of the container for possible shipping damage such as:

- Water damage or discoloration
- Cuts or gashes
- Collapsed corners
- Crushed top, sides, or bottom
- Other physical damage

## Unpacking the instrument

Ensure that there is sufficient space to unpack the instrument and accessories. To unpack the instrument:

1. Remove the plastic wrap, if any, and the bands securing the carton to the pallet. Remove the top and the sides of the shipping carton, and the packing material.
2. Check the contents of each carton against the packing slip to confirm that all items are included.
3. Leave the 2757 Sample Manager in the box and remove the front and side shields from the worktable.
4. Remove the Startup Kit box from the worktable.
5. Check that the contents of the Startup Kit match the Startup Kit list.



**Caution:** To prevent damage, lift the instrument by the bottom. Do not lift the instrument by the robotic arm.

6. Remove the instrument from the bottom styrofoam cushion and the box bottom, and place the instrument on the work surface.



**Caution:** To avoid overheating the 2757 Sample Manager, ensure that there is at least 1 inch (2.5 cm) of clearance at the back of the instrument.

7. Check that the instrument serial number (found on the left side near the power cord connector) corresponds to the number on the Certificate of Validation.
8. Locate the signal cables.

9. If you detect any damage to the contents of any of the cartons, refer to Shipments, Damages, Claims and Returns, in the *Waters Licenses, Warranties, and Support* document.
10. Save the shipping cartons for future transport or shipment.
11. If you detect any discrepancy in the contents of the order, contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

## Releasing the X/Y/Z robotic arm

The X/Y/Z robotic arm is secured to the guard bar for shipping.

### Required material

Scissors

### To release the X/Y robotic arm:

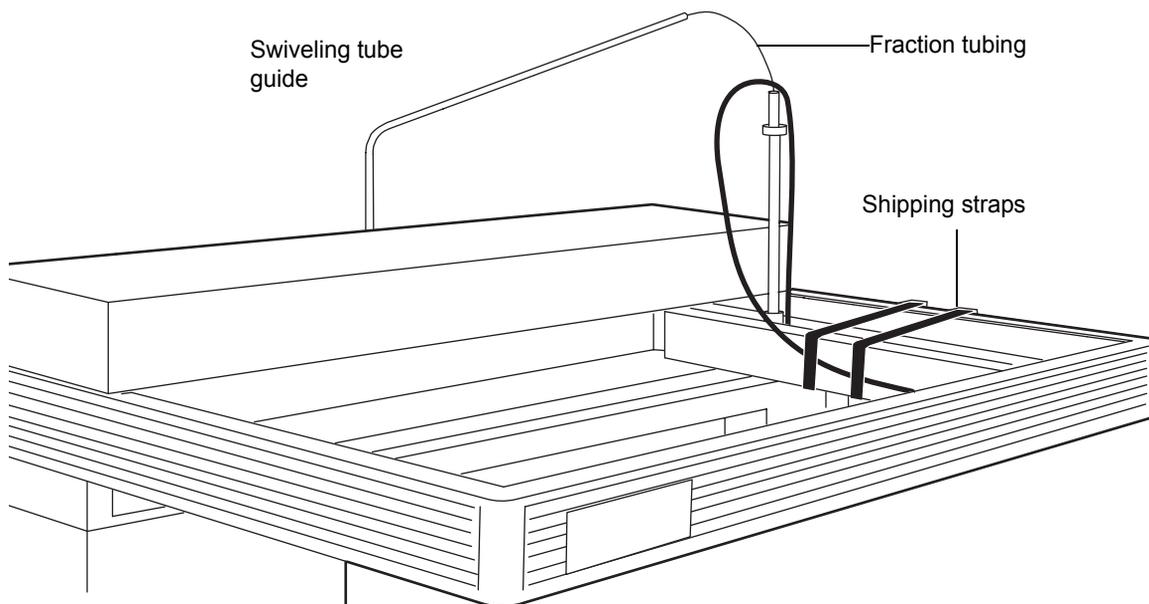
1. Open the Velcro closure that secures the shipping straps around the X/Y robotic arm (see [“Unlocking the X/Y/Z robotic arm” on page 2-7](#)).
2. Remove the packing material from the top of the frame. Remove the tie wrap and tubing from the fraction collect mechanism.



**Caution:** To prevent damage to the X/Y/Z robotic arm when moving, relocating, or shipping the 2757 Sample Manager, reinstall the packing material and secure the robotic arm to the guard bar with the shipping straps before moving the instrument.

3. To release the Z arm, cut tie wrap and remove split clear tubing on top and bottom of each Z drive shaft.

### Unlocking the X/Y/Z robotic arm



### Installing the shields

The 2757 Sample Manager has shields to protect you from injury during operation.



**Warning:** Shields must be installed for safe operation of the 2757 Sample Manager.

**Tip:** If you are installing the fume hood, refer to [“Installing the optional fume hood”](#) on page 2-16. The fume hood includes specialized protective shields.

### Required materials

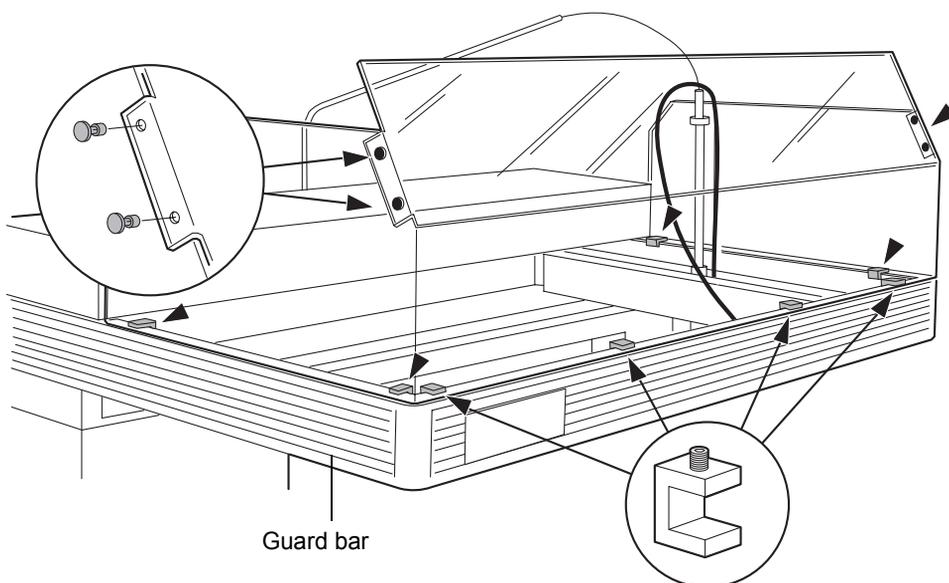
- Side shields (part number 700001391, Startup Kit)
- Front shield (part number 700001390, Startup Kit)
- Screw kit includes clips, screws and black pop-in fasteners (part number 700001433, Startup Kit)

- Allen wrench
- Isopropyl alcohol
- Lint-free cloths

### To install the shields:

1. Remove the paper from the front and side shields.
2. Center the front shield on top of the front guard bar above the worktable. Make sure the flange faces the back of the unit.

### Securing the shields



3. Place the four shield clips with the set screws facing upward onto the flange in the notches. Use an Allen wrench to tighten the set screw in the clamp.



**Caution:** Position the shield clips with set screws facing upward so they do not interfere with the motion of the X/Y/Z arm.

4. Repeat steps 2 and 3 for both side shields using two shield clips per side shield. The side shields are interchangeable.

5. Press the black pop-in fasteners into the holes located at the corners of the front shield to secure it to the side shields. Use two fasteners to secure each side shield to the front shield.
6. Slide the X/Y/Z arm to the far left to ensure that the shields and connectors do not interfere with the movement of the robotic arm.
7. Clean all three shields with isopropyl alcohol and lint-free cloths.

## Making fluidic connections

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Solvents that are compatible with the 2757 Sample Manager can range from water and aqueous solutions to organic solvents. See [Appendix D](#) for limitations. Ensure that the sample and sample matrix are soluble in the solvents that you use for elution and washing.

**Tip:** Mixing aqueous buffers and miscible organic solvents with each other can cause precipitation due to salt formation or solubility limitations. Precipitates can block the fraction dispense tube.

The fluidic connections for the 2757 Sample Manager are as follows:

- Setting up the swiveling tube guide
- Installing fraction collection and HPLC waste tubes
- Installing the wash station drain tube
- Installing the drip tray drain tube



**Caution:**

- Check all plastic fittings and tighten if necessary. The fittings can loosen during shipment, particularly if the unit has undergone temperature extremes.
- For proper drip protection, ensure that the tube fittings are tightened before you operate the system.



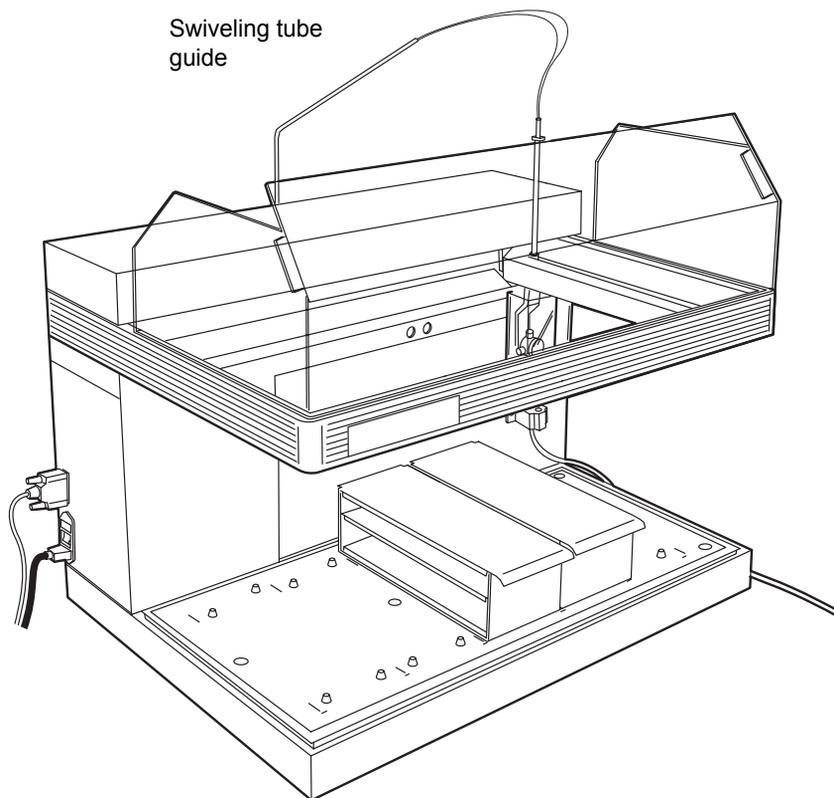
**Warning:** Observe safe laboratory practices when you handle solvents. See the Material Safety Data Sheets for the solvents you use.

## Setting up the swiveling tube guide

The swiveling tube guide gathers the tubes to the fraction dispense tube allowing the X/Y/Z robotic arm to move freely around the worktable.

To set up the swiveling tube guide insert the tube guide into the black holder located on the upper portion of the back panel.

### Setting up the swiveling tube guide



## Installing fraction collection and HPLC waste tubes

### Fraction collection tube

The fraction collection tube connects the fraction dispense tube with the UV detector outlet or flow splitter depending on system configuration. The tube marked T9 (or T27 or T28 from the Startup Kit depending on the desired flow rate range) is prerouted through the swiveling tube guide. Connect the free end of the tube to the detector. The 2757 Sample Manager is equipped with a 0.020-inch ID fraction collection tube (T9). A 0.010-inch ID fraction collection tube (T27) and a 0.040-inch ID fraction collection tube (T28) are also provided

for lower and higher flow rates. Refer to the [table titled “Maximum flow rates for the fraction collection tubes” on page 2-11](#) for the maximum flow rate for each fraction collection tube. Minimizing tubing diameter and length will minimize band spreading.



**Caution:** To prevent tubing or fitting failure, follow the guidelines in the [table titled “Maximum flow rates for the fraction collection tubes” on page 2-11](#).

### Maximum flow rates for the fraction collection tubes

| T number      | Part number | Tube ID (inches) | Maximum flow rate (mL/min) |
|---------------|-------------|------------------|----------------------------|
| T9 (standard) | 700001682   | 0.020            | 30                         |
| T27           | 700001656   | 0.010            | 5                          |
| T28           | 700001657   | 0.040            | 150                        |

### HPLC waste tube

The HPLC waste tube runs from the fraction collect valve to the waste container (not provided) or secondary fraction collector(s) such as the Waters Fraction Collector II.

### Required materials

- Teflon<sup>®</sup> tubing marked T10
- Waste solvent container (compatible with your solvents), not provided

### To install the HPLC waste tube:

1. Locate the tube marked T10, which is prerouted through the swiveling tube guide.
2. Place the free end of the tube in the waste container located below the instrument or secondary fraction collector. Ensure that the HPLC waste tube does not get crimped or bent. A crimp or bend in the line may prevent adequate flow.



**Caution:** To properly drain the HPLC waste fluid, ensure that the end of the HPLC waste tube in the waste container always remains above the level of the waste fluid.

## Installing the wash station drain tube

The 2757 Sample Manager Startup Kit includes one 10-foot Tygon<sup>®</sup> 2075 1/4-inch internal diameter waste tube. You need to cut this tube into two pieces: one piece functions as the wash station drain tube, and the other serves as the drip tray drain tube (see [“Installing the drip tray drain tube” on page 2-13](#)).

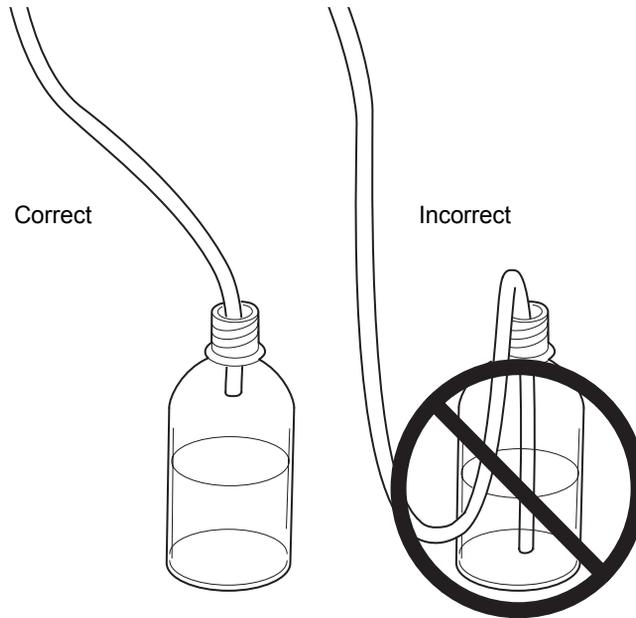
### Required materials

- Tygon 2075 waste tube 1/4-inch internal diameter, 10 feet cut to length (part number 700001428, Startup Kit)
- Waste solvent container (compatible with your solvents), not provided

### To install the wash station drain tube:

1. Cut the waste tube to the length needed to route wash solvent from the wash station to the normal waste bottle (not provided) stored under the 2757 Sample Manager.
2. Connect one end of the tube to the stainless steel fitting on the bottom of the wash station and secure with the hose clamp provided.
3. Place the end of the waste tube into a solvent waste container that is located below the level of the instrument. The wash station drain tube must slope downward to allow the waste fluid to flow into the waste container

## Waste tube configuration



4. Secure the tube to the container. Loosely cover the waste container opening with laboratory film to prevent fumes and splashes without sealing it tightly.



**Caution:** To properly drain the waste fluid, ensure that the:

- End of the waste station drain tube in the waste container always remains above the level of the waste fluid.
- Waste station drain tube does not get crimped or bent. A crimp or bend in the line may prevent adequate flow to the waste container.
- Waste station drain tube has a downward slope of 1/2 inch per foot to provide adequate drainage.

## Installing the drip tray drain tube

The 2757 Sample Manager is equipped with a drip tray to collect any liquid spill in the worktable area. The drip tray has a separate drain tube to an auxiliary waste container (not provided) or the optional leak sensor module.

This drain tube isolates any liquid that may overflow into the drip tray from the normal waste. In normal operation solvent should not flow into the drip tray.

The 2757 Sample Manager Startup Kit includes one 10-foot Tygon 1/4-inch ID waste tube. You need to cut this tube into two pieces: one piece functions as the wash station drain tube (see [“Installing the wash station drain tube” on page 2-12](#)), and the other serves as the drip tray drain tube.

## Required materials

- Tygon 2075 waste tube 1/4-inch internal diameter, 10 feet cut to length (part number 700001428, Startup Kit)
- Waste solvent container (compatible with your solvents), not provided
- Optional leak sensor module

## To install the drip tray drain tube:

1. Affix the Tygon (1/4-inch ID) drain tube to the drain port located on the bottom-right side of the drip tray.
2. Place the end of the drip tray drain tube into the fluid waste container or optional leak sensor module that is located below the level of the instrument. The drip tray drain tube must slope downward to allow the waste fluid to flow into the waste container (see the [figure “Waste tube configuration” on page 2-13](#)).
3. Label the waste container as drip tray waste.
4. Secure the tube to the waste container. Cover the waste container opening with laboratory film to prevent fumes and splashes without sealing it tightly.



**Caution:** To properly drain the waste fluid, ensure that the:

- End of the drip tray drain tube in the waste container always remains above the level of the waste fluid.
- Drip tray drain tube does not get crimped or bent. A crimp or bend in the line may prevent adequate flow to the waste container.
- Drip tray drain tube has a downward slope of 1/2 inch per foot to provide adequate drainage. Do not insert a trap on the drip tray drain tube.

## Making electrical connections

---

Use this section to:

- Install the communications cable
- Make the power connection

### Installing the communications cable

#### Required material

- DB9 RS-232 cable kit (includes DB25 male to DB9 female adapter, part number 700001408, Startup Kit, see the [figure “Major hardware components” on page 1-4](#))

**To install the RS-232 communications cable from the 2757 Sample Manager to the computer:**

1. Connect the 25-pin male side of the DB25 male to DB9 female adapter to the 25-pin D-subreceptacle located on the left side of the 2757 Sample Manager (see the [figure “Major hardware components” on page 1-4](#)), then tighten into position.
2. Connect the male side of the standard 9-pin RS-232 cable to the 9-pin female side of the adapter.
3. Connect the other side of the RS-232 cable (female side) to the 9-pin male serial port connector, on the back of your computer, then tighten into position.

### Making power connections

The 2757 Sample Manager uses two 5-A main fuses (see [Appendix B, Specifications](#)). The power supply automatically switches configuration for your power source (110/220 Vac).



**Warning:** To avoid possible electrical shock, always power off the instrument before you connect or disconnect the power cord.

**To install the power cord from the 2757 Sample Manager to the power source:**

1. Attach the female connector into the power receptacle on the left side of the instrument.
2. Connect the male connector into an earth-grounded power source (see [Appendix B, Specifications](#)).

## **Installing the optional fume hood**

---

The 2757 Sample Manager can be equipped with an optional fume hood to allow for self-contained ventilation.

### **Required materials**

- Fume hood (part number 205000125)
- Screw kit includes clips, screws, and black pop-in fasteners (part number 700001433, Startup Kit)
- Allen wrench
- Isopropyl alcohol
- Lint-free cloths
- Duct work (4-inch), not supplied

### **To install the fume hood:**

1. Remove the paper from the top shield of the fume hood.
2. Mount the top shield of the fume hood on the guard bar above the worktable.
3. Lift the metal rear portion of the fume hood to align the side channels with the side flanges of the front cover. When the side channels and flanges are aligned, lower the metal rear portion of the fume hood until it rests on top of the 2757 Sample Manager.
4. Slide the fume hood forward so that the front edge aligns with the guard bar.
5. Install eight shield clips with the set screws facing upward along the guard bar to secure the fume hood. There are four notches in the front of

the guard bar and two notches on each side of the guard bar for the shield clips. Use an Allen wrench to tighten the set screw in the clamp.

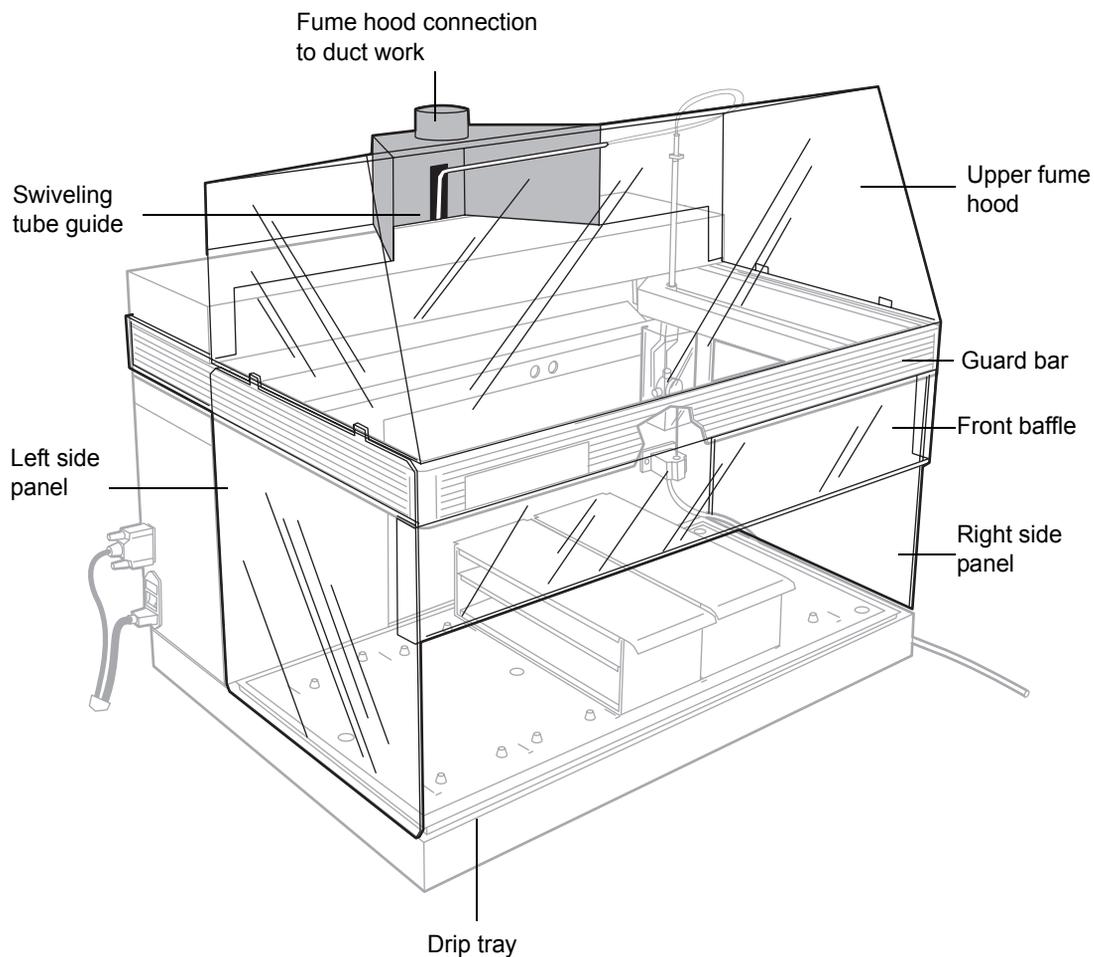
6. Ensure that the swiveling tube guide pivots freely inside the fume hood. If the swiveling tube guide rubs the inside the fume hood, bend the swiveling tube guide at the elbow slightly. The movement of the swiveling tube guide must not be restricted.
7. Hold the right side panel with the flange toward the worktable of the 2757 Sample Manager and align the top with the slots in the upper fume hood. Carefully bend the side panel slightly to lift the side panel over the drip tray so the flange rests inside the drip tray.
8. Repeat step 7 for the left side panel.
9. Place the front baffle under the front guard bar and secure it to the side panels with the black pop-in fasteners.



**Caution:** Position the shield clips with set screws facing upward so they do not interfere with the motion of the X/Y/Z arm.

10. Clean all shields with isopropyl alcohol and lint-free cloths.
11. Connect the duct work (not provided) to the opening on the metal rear portion of the fume hood. The 4-inch duct work must provide greater than 80 CFM flow with a minimum face velocity of 10 feet per minute.

## Installing the fume hood



## Setting up the MassLynx software

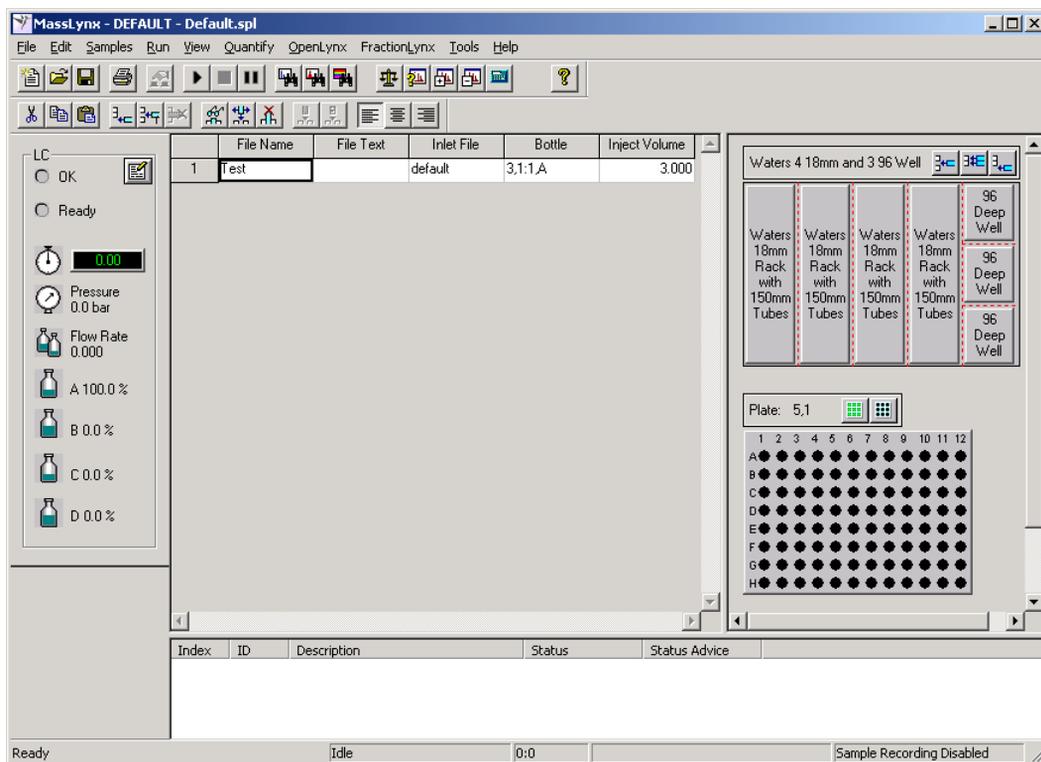
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The 2757 Sample Manager is controlled by MassLynx software version 3.5x or higher with appropriate Software Change Notes. Refer to the *MassLynx User's Guide* for information on selecting and displaying data files.

## Configuring the software

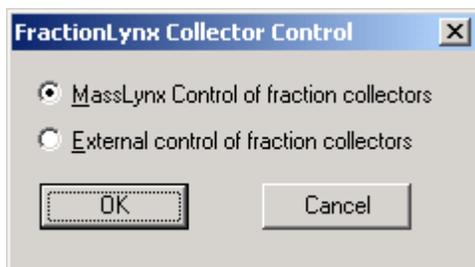
The MassLynx Main window displays three panes of information. The left pane displays instrument status, the middle pane displays sample information, and the right pane displays bed layout and plate information of the rack selected for sample injection, not the fraction collection bed layout.

### MassLynx main window



From the MassLynx Main window, select **FractionLynx > FractionLynx Collector Control**. The FractionLynx Collector Control dialog box below appears.

## Setting MassLynx collector control

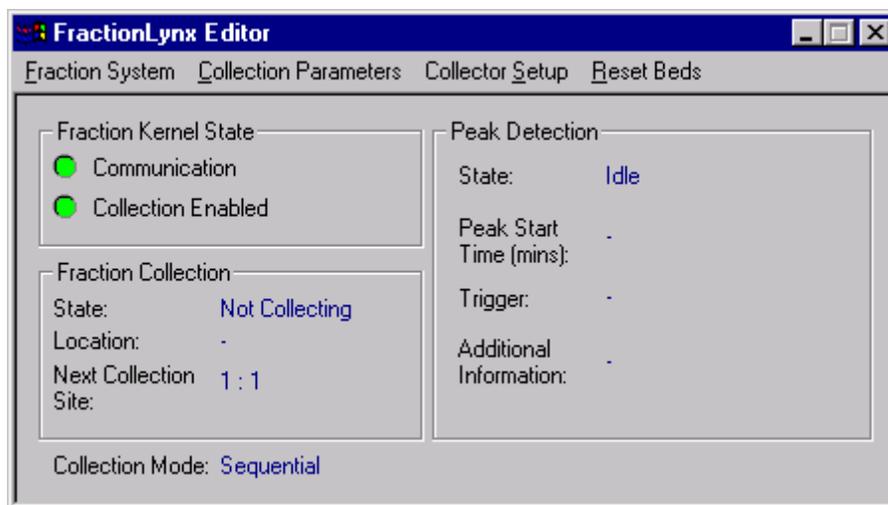


Click **MassLynx Control of fraction collectors**, then click **OK**.

To enable FractionLynx control of the fraction collector, select **FractionLynx > FractionLynx Editor** from the MassLynx Main window to open the FractionLynx Editor shown below.

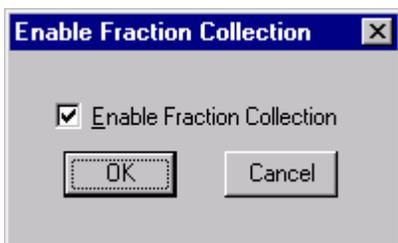
**Tip:** The FractionLynx Editor must remain open while you are collecting fractions.

## FractionLynx editor window



From the FractionLynx Editor, select **Fraction System > Enable Fraction Collection**. The Enable Fraction Collection dialog box below appears. Select **Enable Fraction Collection**, then click **OK** to close the dialog box.

## Enabling fraction collection



To designate the 2757 Sample Manager as the collection instrument, select **Collector Setup > Instrument Setup** from the FractionLynx Editor. The Fraction Collection Setup dialog box appears.

## Selecting the 2757 Sample Manager



## Instrument Configuration page

The Instrument Configuration page details the type and number of fraction collector(s) configured in your system.

## Number Of Collectors

Displays the number of fraction collectors configured in your system. The range is 1 to 9, with 1 as the default.

## Fraction Collector

Displays which fraction collector parameters are active in a system with several fraction collectors. The default is 1.

## Collector Model

Displays the fraction collector model configured in your system. The default is Waters FCII but you must select **Waters 2757** from the list.

## 2757 Setup

Displays the communications port used for the RS-232 communications cable. The 2757 Sample Manager can be configured to operate using any RS-232 communications port. The default setting is COM1.

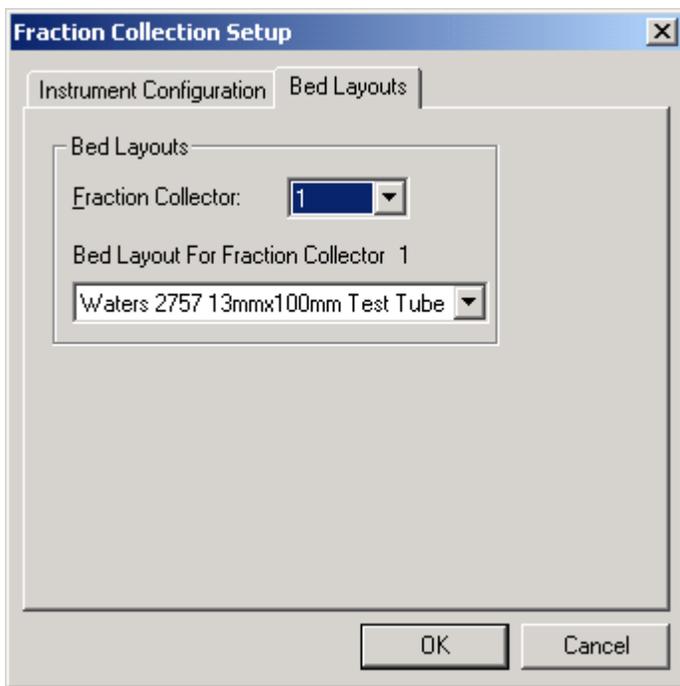
## Collection Type

Specifies the collector function: fraction collector or waste collector. The 2757 Sample Manager is typically configured as a fraction collector, so select **Collect Fractions**.

## Bed Layouts page

The Bed Layouts page shown below designates the bed layout used with the selected fraction collector. Select **Waters 2757 13mm×100mm Test Tube Bed** from the Bed Layout For Fraction Collector 1 list. In this example, fraction collector 1 is equipped with the bed layout entitled Waters 2757 13mm×100mm Test Tube Bed.

## Selecting the bed layout for fraction collection



If you have multiple fraction collectors using the same bed layout confirm that the x-, y-, and z-coordinates are correct. X-, y-, and z coordinate information is stored with the bed layout on the Plate Description dialog box (see the [figure “Plate description dialog box” on page 2-35](#)). Since the range of travel and x-, y-, and z-coordinates may vary among different types of fraction collectors it may be necessary to save similar bed layouts with distinct names. For information on creating or modifying the bed layout, refer to [“Configuring the bed layout” on page 2-30](#).

**Tip:** You must select the “Waters 2757 13 mm×100mm Test Tube Bed” bed layout or another bed layout configured for the 2757 Sample Manager. The default bed layout, Fraction 120 Test Tubes, is optimized for a different Waters fraction collector.

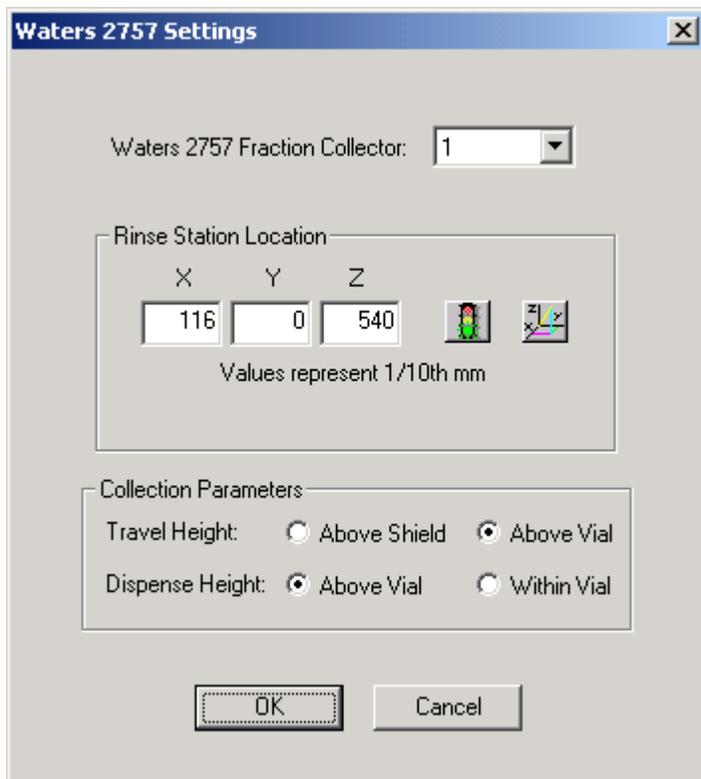
**Tip:** If you have multiple fraction collectors, the bed layout for every fraction collector must be selected before leaving the Fraction Collection Setup dialog box.

After selecting the bed layout for each fraction collector, click **OK** to close the dialog box and reinitialize the instrument.

## Configuring the 2757 Sample Manager

The parameters specific to the 2757 Sample Manager are set on the Waters 2757 Settings dialog box. Select **Collector Setup > Waters2757 Settings** to access the Waters 2757 Settings dialog box.

### Setting 2757 Sample Manager parameters



The Waters 2757 Fraction Collector drop-down list displays the 2757 Sample Managers connected to your system. This list does not include any other type of fraction collector that is part of your system configuration.

The Rinse Station Location area displays the x-, y-, and z-coordinates of the rinse station (see the [figure “2757 Sample Manager fluidic path” on page 1-3](#)). To check the rinse station position, see [“Verifying the rinse station location” on page 2-25](#).

The Collection Parameters area specifies the travel height and the dispense height of the fraction dispense tube during operation.

- **Travel Height:** Selecting **Above Shield** retracts the tip of the fraction dispense tube to the lower edge of the safety shield enclosing the fraction dispense mechanism before moving to the next location. Selecting **Above Vial** positions the fraction dispense tube at a height of 4 mm above the top plane of the collection vessel before moving to the next location. The top plane of the collection vessel is defined by the height specified for the rack in the bed layout when teaching the rack, see [“Teaching racks or containers” on page 2-36](#). For optimum speed, click the **Above Vial** option button. The default is Above Vial.
- **Dispense Height:** Selecting **Above Vial** positions the tip of the fraction dispense tube at a height of 4 mm above the top plane of the collection vessel before collecting the fraction. The top plane of the collection vessel is defined by the height specified for the rack in the bed layout when teaching the rack, see [“Teaching racks or containers” on page 2-36](#). The Above Vial option button provides the most rapid movement from vial to vial. Selecting **Within Vial** lowers the tip of the fraction dispense tube to 4 mm within the vial to reduce fraction loss due to static spray and prevent possible cross contamination. The default setting is Above Vial.

**Tip:** Do not cover the vessels in the fraction collection racks. The vessels must be open so the fraction dispense tube can deliver the fraction.

## Verifying the rinse station location

---

The fraction dispense tube requires checking to ensure that it is centered at the rinse location:

- Before first use
- When the fraction dispense tube is replaced
- If the movement of the fraction dispense tube has been impeded



**Warning:** To avoid possible injury, always keep your hands outside the worktable and do not lean over the instrument during this procedure.

The rinse station location is the home position for the 2757 Sample Manager.

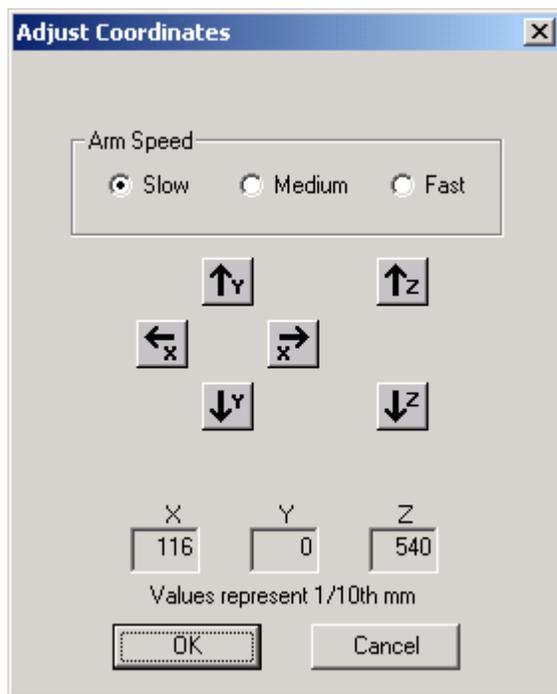
### To check the rinse station location:

1. From the FractionLynx Editor, select **Collector Setup > Waters2757 Settings**. The Waters 2757 Settings dialog box (see the [figure “Setting 2757 Sample Manager parameters” on page 2-24](#)) appears.

2. Click  (Adjust coordinates) to access the Adjust Coordinates dialog box, shown below.

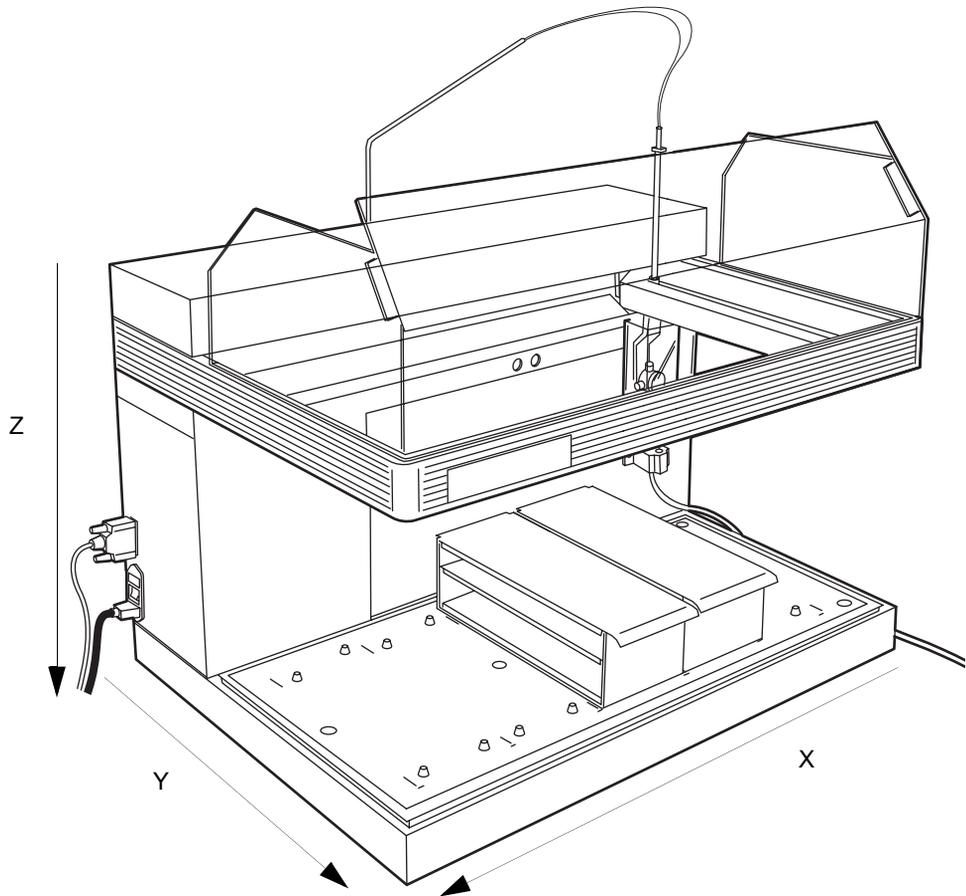
**Tip:** If you know the x-, y-, and z-coordinates, you can enter the numbers in the Waters 2757 Settings dialog box. You cannot enter numbers in the Adjust Coordinates dialog box.

### Adjusting the coordinates



The x-, y-, and z-coordinates are measured in 1/10 millimeters, so 1 mm equals 10 units. The [figure “Travel directions of the fraction dispense tube” on page 2-27](#) shows the x-, y-, and z-directions with the arrowheads pointing in the direction of increasing numbers for the coordinates. The number of steps per move varies according to whether Slow, Medium, or Fast is selected, with Slow having the least and Fast having the most steps per move.

## Travel directions of the fraction dispense tube



3. Click the arrows in the Adjust Coordinates dialog box or use the arrow keys (right and left for the x-direction, up and down for the y-direction, or Page Up and Page Down for the z-direction) to move the fraction dispense tube to the rinse station. Select an arm speed appropriate for the amount of movement required.
4. Click **OK** when you are satisfied with the rinse station position. The Waters 2757 Settings dialog box returns.
5. Click  (Move to) to verify the position.
6. Click **OK** to save the settings.

## Setting up the worktable

---

Setting up worktables involves customizing the worktables for your fraction collection racks. The 2757 Sample Manager uses rectangular racks that hold test tubes, vials, or deep-well microplates. You position the racks horizontally in the worktable. The bed layout in the Main MassLynx window designates the arrangement and the types of racks that hold test tubes, vials, or deep-well microplates. The 2757 Sample Manager worktable holds up to five racks. Setting up a worktable can include any of the following procedures:

- Selecting a rack
- Loading racks into the worktable
- Configuring the bed layout
- Teaching the racks and containers
- Changing a rack in a worktable
- Customizing a rack

After customizing the bed layout, you must calibrate the first position of each rack (see [“Teaching racks or containers” on page 2-36](#)).

### Selecting a rack

Fraction collection racks do not use a stripper plate so the test tubes, vials, or plates must not be covered. If you are using microplates, use only 96-deep well microplates for fraction collection.

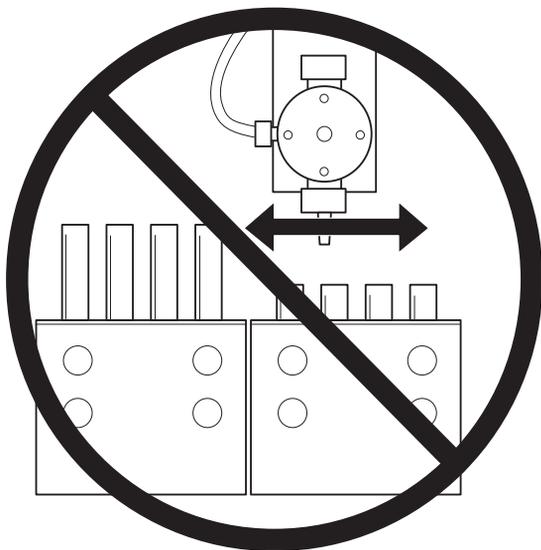
Use the following racks for fraction collection:

- **13-mm tubes** – 112-test tube rack for 13 × 100 mm test tubes
- **16-mm tubes** – 84-test tube rack for 16 × 100 to 150 mm test tubes
- **18-mm tubes** – 78-test tube rack for 18 × 150 mm test tubes
- **25-mm tubes** – 36-test tube rack for 25 × 150 mm test tubes
- **28-mm scintillation vials** – 32-vial rack for 28-mm diameter scintillation vials
- **MTP deep** – Racks hold three 96-well deep format microtiter plates

When the 2757 Sample Manager collects fractions, the Z-arm lowers the fraction dispense tube to the setting specified on the Waters 2757 Settings dialog box (see the [figure “Setting 2757 Sample Manager parameters” on](#)

page 2-24). In placing fraction collection racks in the worktable, put the fraction collection rack containing the taller test tubes to the right of any fraction collection rack with shorter test tubes.

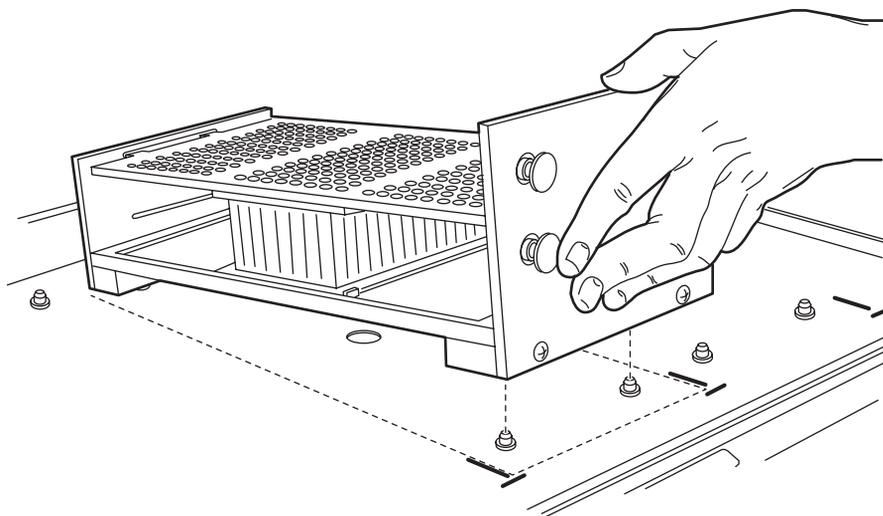
### **Positioning fraction collection racks with different test tube heights in the worktable**



### **Loading racks into the worktable**

Line up the racks using the marks on the worktable, then lower the back end of the rack and slide it toward the back of the worktable. The notches on the worktable fit into the bottom of the rack to locate it. The long marks on the worktable outline the rectangular area of the Waters racks.

## Placing a rack on the worktable



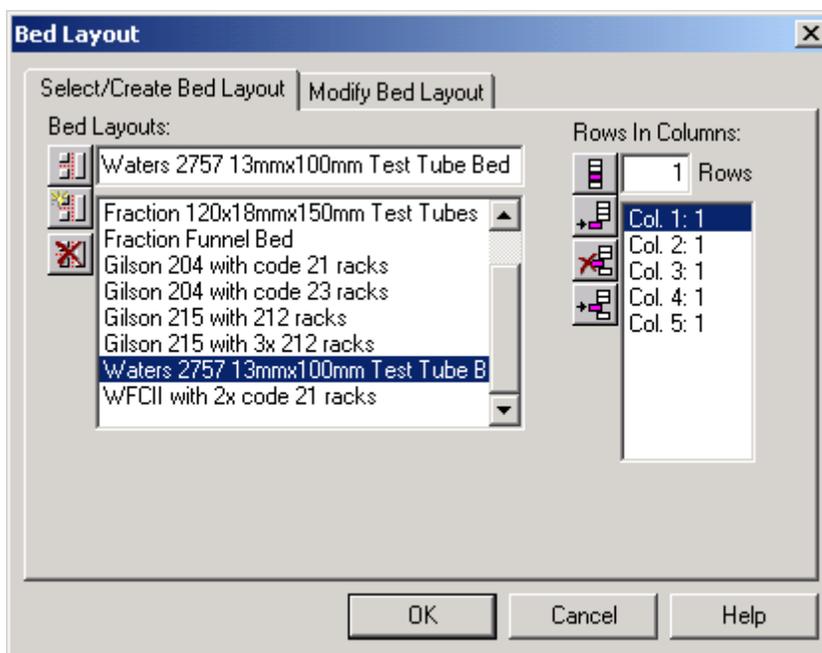
Racks manufactured by Gilson<sup>®</sup> can be used for fraction collection. When placing Gilson racks onto the worktable use the small circular marks to position the curved front of the rack.

## Configuring the bed layout

The bed layout graphically displays the types and relative location of rack used in the 2757 Sample Manager worktable. You configure the bed layout using the Bed Layout dialog box. You can also use this dialog box to change existing bed layouts or customize new layouts.

To configure your bed layout, select **Collector Setup > Bed Layout Editor** from the FractionLynx Editor (see the [figure “FractionLynx editor window” on page 2-20](#)). The Bed Layout dialog box appears.

## Bed layout dialog box



### Select/create bed layout page

This page displays the bed layout that you are editing, and a list of other defined bed layouts. The Bed Layouts field displays the bed selected for editing. The bed layout is arranged in columns and rows and describes the racks or plates on the worktable. Racks in the bed layout are numbered from left to right. The Rows In Columns section of the screen displays the number of columns and rows in the bed being edited. In this example, the Waters 2757 13mm×100mm Test Tube Bed shows test tubes in columns 1 to 5 (Col. 1:1, Col. 2:1, Col. 3:1, Col. 4:1, and Col. 5:1). The table below describes the function of each icon in the Select/Create Bed Layout page.

## Select/create bed layout icons

| Icon  | Description  |
|---|--|
| <b>Bed Layouts</b>  |  |
| <br>(Change name of selected layout)           | Changes the name of the selected bed layout when you type in a new name.   |
| <br>(New layout)                               | Creates a new bed layout.  |
| <br>(Remove selected layout)                   | Deletes the selected bed layout.   |
| <b>Rows In Columns</b>  |  |
| <br>(Change number of rows in current column) | Causes the number of rows in the selected column to be equal to the number displayed in the Rows field.                    |
| <br>(Append new column to current layout)    | Adds a new column to the end of the bed layout. This column contains the same number of rows as shown in the Row edit box. |
| <br>(Delete current column)                  | Deletes the currently selected column.   |

## Select/create bed layout icons (Continued)

| Icon  | Description  |
|---|--|
| <br><br>(Insert column at current list location) | Inserts a column above the currently selected column. This column contains the same number of rows as shown in the Row edit box. On the worktable, the inserted column appears to the left of the initially selected column. |

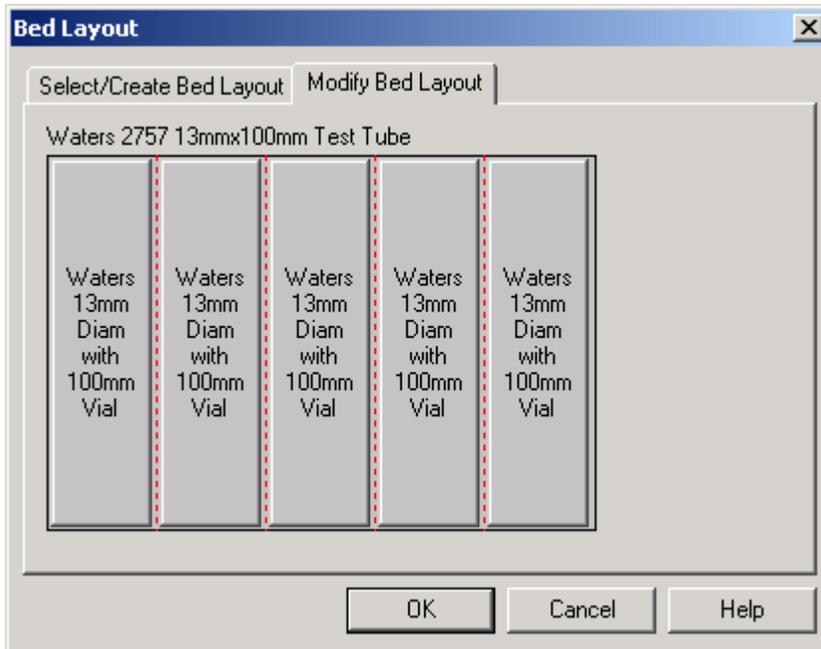
### To create a new bed layout:

1. Type a descriptive name in the Bed Layouts field and click  (New layout) to create a new bed layout.
2. Enter the number of rows in the rack in the Rows In Columns field. For bed layout purposes, columns refer to the number of racks in the worktable and rows refer to microplates in a rack. A rack with three microplates contains three rows while a rack with test tubes contains one row. The number of test tubes in a rack or wells in a plate is defined in the Rack Generator window (see the [figure “Rack generator window” on page 2-39](#)).

### Modify bed layout page

This page displays a graphical representation of the bed layout selected for editing. This example shows five racks with 13 × 100 mm tubes in the worktable.

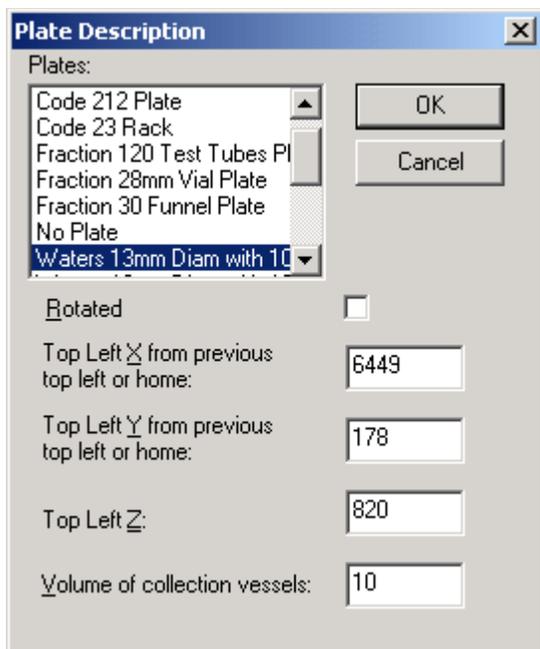
## Modify Bed Layout page



### Plate Description

Click a rack (Waters 13mm Diameter with 100mm Vial) in the Modify Bed Layout page to access the Plate Description dialog box (see the [figure “Plate description dialog box” on page 2-35](#)). These racks are created and defined using the Rack Generator window (see the [figure “Rack generator window” on page 2-39](#)).

## Plate description dialog box



### Plates

The plate or rack highlighted is the plate or rack you selected from the Modify Bed Layout page. All information on the Plate Description dialog box refers to the selected plate or rack. To select another rack or plate, scroll through the list and highlight the desired plate or rack type.

### Rotated

The 2757 Sample Manager does not utilize the rotated check box.

Top Left X from previous top left or home and Top Left Y from previous top left or home

Displays the x- and y-coordinates in 1/10 mm from the origin to the top-left vial or test tube position in the rack.

### Top Left Z

Displays the z-coordinate that places the tip of the fraction dispense tube at the top plane of the vial or test tube in top-left position. One z-coordinate is set for each plate.

## Volume of collection vessels

Displays the maximum volume that each collection vessel holds. The [table titled “Maximum flow rates for the fraction collection tubes” on page 2-11](#) lists the maximum volumes for common collection vessels.

### Nominal maximum volume of collection vessels

| Collection vessel           | Volume (mL) |
|-----------------------------|-------------|
| 28 mm × 95 mm Vial          | 43.0        |
| 28 mm × 57 mm Vial          | 25.0        |
|                             |             |
| 25 mm × 150 mm Test tube    | 57.0        |
| 18 mm × 150 mm Test tube    | 30.0        |
| 16 mm × 150 mm Test tube    | 23.0        |
| 16 mm × 125 mm Test tube    | 19.0        |
| 16 mm × 100 mm Test tube    | 15.0        |
| 13 mm × 100 mm Test tube    | 10.0        |
|                             |             |
| 96 Deep well microplate     | 2.4         |
|                             |             |
| WISP vial 24 position plate | 4.0         |
| WISP vial 48 position plate | 2.0         |

## Teaching racks or containers

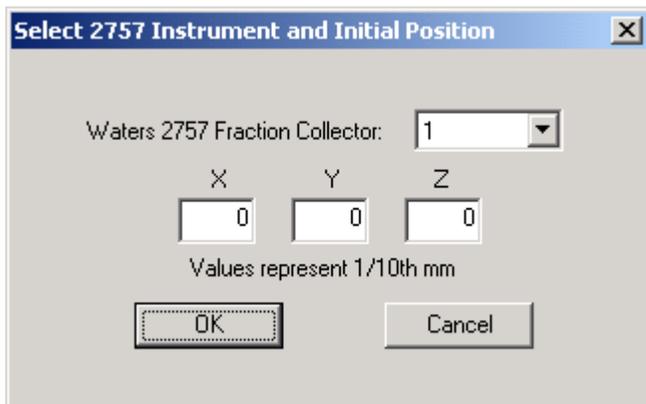
After placing your racks in the worktable and setting up the worktable using MassLynx software, you must calibrate the fraction dispense tube to the top-left position in each rack and teach the fraction dispense tube the top plane of your collection container. The x-, y-, and z-coordinates for each plate are stored in the Plate Description dialog box (see the [figure “Plate description dialog box” on page 2-35](#)).

### To teach racks or containers:

1. From the FractionLynx Editor window (see the [figure “FractionLynx editor window” on page 2-20](#)), select **Collector Setup > Waters 2757**

**Plate Positioning Guide.** The Select 2757 Instrument and Initial Position dialog box appears.

### Teaching racks and containers



2. Ensure that the correct fraction collector appears in the Waters 2757 Fraction Collector field.
3. Click **OK** to move to the fraction dispense tube to the upper-right back corner of the worktable. The Adjust Coordinates dialog box (see the [figure “Adjusting the coordinates” on page 2-26](#)) appears. Alternatively, you can enter x-, y-, and z-coordinates, if known. The table below displays the maximum coordinate values.

### Maximum coordinate values

| Axis | Maximum value |
|------|---------------|
| x    | 6449          |
| y    | 2998          |
| z    | 1699          |

**Tip:** If the 2757 Sample Manager arm is impeded during travel, an error message identifies which axis encountered difficulty. To clear the error message and resume operation, select **Fraction System > Reset Communications** from the FractionLynx Editor.

4. Click the arrows in the Adjust Coordinates dialog box or use the arrow keys (right and left for the x-direction, up and down for the y-direction, or Page Up and Page Down for the z-direction) to move the fraction

dispense tube to the top-left position of the rack. Select an arm speed appropriate for the amount of movement required. Lower the fraction dispense tube until the tip of the tube is at the top plane of your collection vessel. Leave the Adjust Coordinates dialog box open.

5. Select **Collector Setup > Bed Layout Editor** from the FractionLynx Editor. Click **Modify Bed Layout** to access the Modify Bed Layout page (see the [figure “Modify Bed Layout page” on page 2-34](#)).
6. Click the appropriate rack to access the Plate Description dialog box (see the [figure “Plate description dialog box” on page 2-35](#)).
7. Enter the x-, y-, and z-coordinates listed in the Adjust Coordinates dialog box in the Plate Description dialog box.
8. Repeat steps 6 and 7 for each rack in the bed layout.
9. Click **OK** in the Plate Description dialog box to save the settings.
10. Click **OK** in the Adjust Coordinates dialog box to close it.

**Tip:** The Adjust Coordinates dialog box must be closed before running a sample set.

## Changing a rack in a worktable

To change the plate type in a previously defined bed layout, you must modify that layout.

### To modify an existing bed layout:

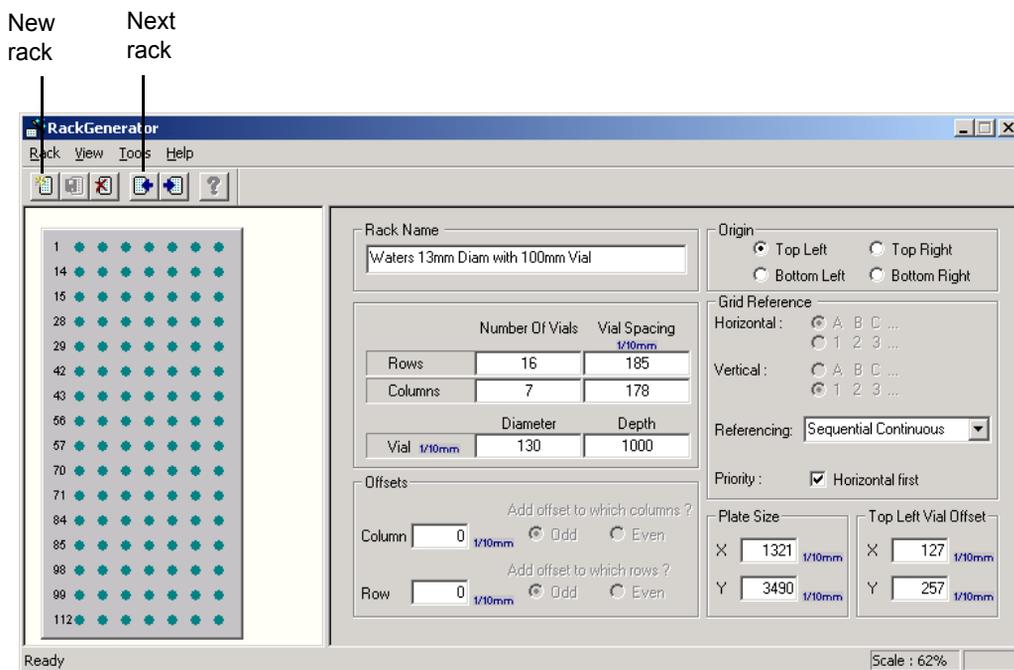
1. From the FractionLynx Editor (see the [figure “FractionLynx editor window” on page 2-20](#)), select **Collector Setup > Bed Layout Editor**. The Bed Layout dialog box (see the [figure “Bed layout dialog box” on page 2-31](#)) appears, displaying a list of defined bed layouts.
2. Select the layout you want to modify from the Bed Layouts list, then click **Modify Bed Layout**. The Modify Bed Layout page appears (see the [figure “Modify Bed Layout page” on page 2-34](#)). Click the plate that you want to change. The Plate Description dialog box appears (see the [figure “Plate description dialog box” on page 2-35](#)).
3. Select the plate you want to use to replace the selected plate in the bed layout.

4. Teach the top left x-, y-, and z-position for the plate, if necessary (see “Teaching racks or containers” on page 2-36).
5. Click **OK** to save your selection and close the dialog box.

## Customizing a rack

The Rack Generator window allows you to define each rack or plate. To customize a plate or rack type, select **Collector Setup > Plate Generator** from the FractionLynx Editor window (see the figure “FractionLynx editor window” on page 2-20). The Rack Generator window appears.

### Rack generator window



To create a new rack, select **Rack > New Rack** or click  (New Rack). To modify an existing rack, click  (Next Rack) to scroll through the list of defined racks until the rack appears in the Rack Name text box.

The graphic on the left portion of the window shows the current plate or rack.

**Tip:** Save your plate or rack by selecting **Rack > Save Current Rack**.

## Rack name

Displays the rack or plate being edited. In this example, Waters 13mm Diam with 100mm Vial.

## Number Of vials

Enter the vials in rows (horizontal) and columns (vertical) that make up the plate or rack. In this example, the 96-deep well plate has 8 rows and 12 columns.

## Vial spacing

Enter the distance from the center of one vial to the center of the next vial in 1/10 mm.

## Diameter

Enter the vial diameter in 1/10 mm.

## Depth

Enter the vial depth in 1/10 mm.

## Offsets

Use the offset parameters if the rows and columns in your rack are not aligned in straight lines. The offset measurements are in 1/10 mm. Waters racks do not use offsets and the default values are 0.

## Origin

Click the option button that represents the location of the first vial in your rack or plate. In this example, the first vial, 1A, is positioned at the top left of the plate.

## Grid referencing

The Grid Reference feature allows you to define up to 12 vial referencing schemes for your racks. Each referencing scheme defines the labeling and sequencing of the vials or wells in the rack. You can use XY, sequential continuous, or sequential discontinuous referencing, assign horizontal or vertical priority, and (for XY sequencing) define the horizontal and vertical

axes as either numerical or alphabetic. The [table titled “Grid reference parameters” on page 2-41](#) describes the Grid Reference parameters in the Rack Generator window (see the [figure “Rack generator window” on page 2-39](#)). For more information, see the next section [“Vial referencing examples” on page 2-41](#).

### Grid reference parameters

| Parameter              | Description  |
|------------------------|--|
| Horizontal             | Specifies the horizontal axis positions as either alphabetic (ABC) or numeric (123), when using XY referencing. Default: numeric.  |
| Vertical               | Specifies the vertical axis positions as either alphabetic (ABC) or numeric (123), when using XY referencing. Default: alphabetic.   |
| Referencing            | Specifies the vial referencing scheme for the current rack. Possible values are XY, Sequential Discontinuous, and Sequential Continuous (see the <a href="#">figure “Vial referencing examples” on page 2-42</a> ). Default: XY.   |
| <b>Priority</b>        |  |
| XY Referencing         | With Horizontal first selected, specifies that the horizontal axis position appears first when referencing a vial (1,A). When Horizontal first is not selected, specifies that the vertical axis position appears first when referencing a vial (A,1). See the <a href="#">figure “Vial referencing examples” on page 2-42</a> for examples. Default: Horizontal first selected. |
| Sequential Referencing | With Horizontal first selected, specifies that vials be numbered horizontally (left to right). When Horizontal first is not selected, specifies that vials be numbered vertically (top to bottom). See the <a href="#">figure “Vial referencing examples” on page 2-42</a> for examples. Default: Horizontal first selected.   |

### Vial referencing examples

The following figure shows four examples of vial referencing for a simplified 4 × 3 vial rack.

## Vial referencing examples

|          | 1   | 2   | 3   | 4   |  |
|----------|-----|-----|-----|-----|--|
| <b>A</b> | 1,A | 2,A | 3,A | 4,A | <b>Horizontal: 1 2 3...</b><br><b>Vertical: A B C...</b><br><b>Referencing: XY</b><br><b>Priority: Horizontal first selected</b> |
| <b>B</b> | 1,B | 2,B | 3,B | 4,B |  |
| <b>C</b> | 1,C | 2,C | 3,C | 4,C |  |

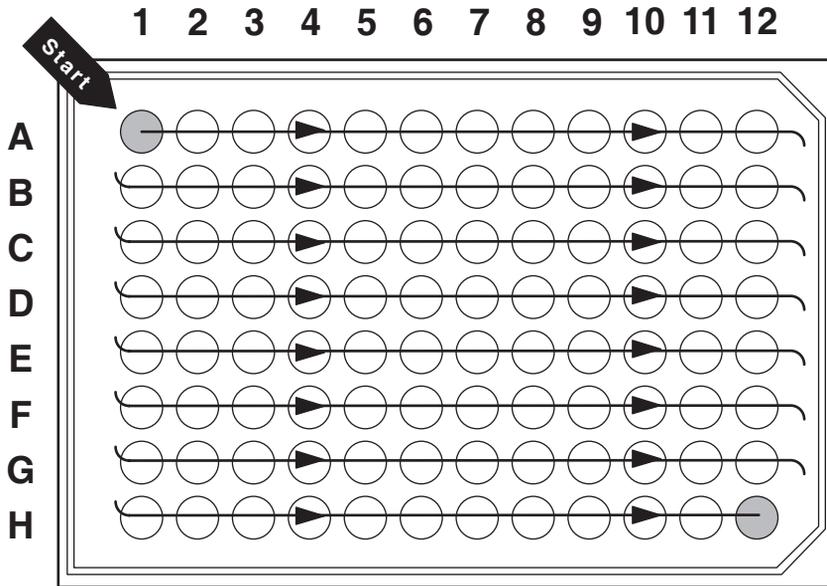
|          | 1   | 2   | 3   | 4   |   |
|----------|-----|-----|-----|-----|---|
| <b>A</b> | A,1 | A,2 | A,3 | A,4 | <b>Horizontal: 1 2 3...</b><br><b>Vertical: A B C...</b><br><b>Referencing: XY</b><br><b>Priority: Horizontal first <i>not</i> selected</b> |
| <b>B</b> | B,1 | B,2 | B,3 | B,4 |   |
| <b>C</b> | C,1 | C,2 | C,3 | C,4 |   |

|  | 1 | 2  | 3  | 4  | <b>Horizontal: N/A</b><br><b>Vertical: N/A</b><br><b>Referencing: Sequential discontinuous</b><br><b>Priority: Horizontal first selected</b> |
|--|---|----|----|----|--|
|  | 5 | 6  | 7  | 8  |  |
|  | 9 | 10 | 11 | 12 |  |

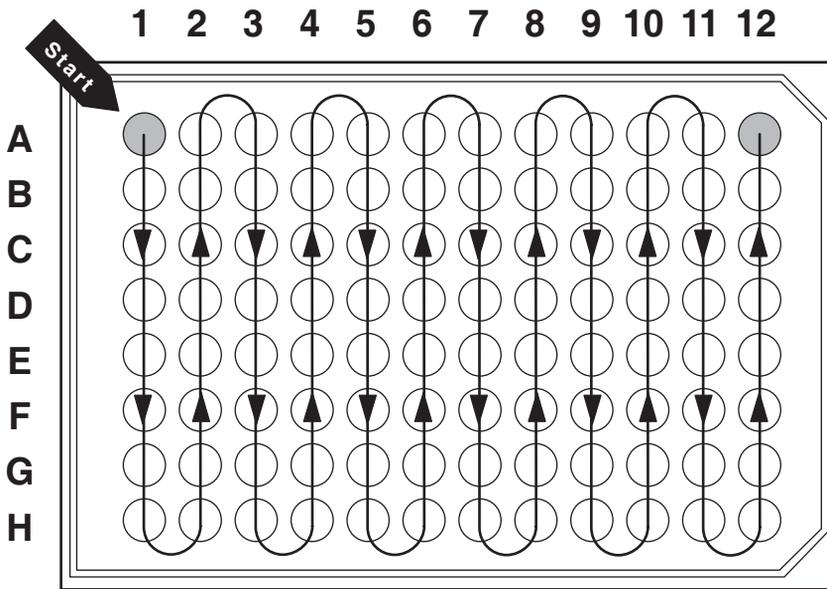
|  | 1 | 6 | 7 | 12 | <b>Horizontal: N/A</b><br><b>Vertical: N/A</b><br><b>Referencing: Sequential continuous</b><br><b>Priority: Horizontal first <i>not</i> selected</b> |
|--|---|---|---|----|--|
|  | 2 | 5 | 8 | 11 |  |
|  | 3 | 4 | 9 | 10 |  |

The two figures below show a sequential processing order for the last two examples in the [table titled “Vial referencing examples” on page 2-42](#), on a 96-well microplate.

**Sequential discontinuous horizontal first vial referencing**



**Sequential continuous nonhorizontal first vial referencing**



## **Plate size**

The 2757 Sample Manager does not use the Plate Size fields. Default values may appear in these fields to support other instruments.

## **Top left vial offset**

The 2757 Sample Manager does not use the Top Left Vial Offset fields. Default values may appear in these fields to support other instruments.

# 3 Preparing for Operation

This chapter describes how to prepare the Waters 2757 Sample Manager for operation.

## Contents

| Topic                              | Page |
|------------------------------------|------|
| Setting MassLynx method parameters | 3-2  |
| Collecting fractions               | 3-2  |

## Setting MassLynx method parameters

---

Setting up a MassLynx method involves establishing run-specific conditions. Select the appropriate inlet method for the application, refer to the *FractionLynx User's Guide*.

## Collecting fractions

---

Setting up fraction collection using the 2757 Sample Manager involves:

- Setting fraction collection parameters
- Setting plate parameters
- Setting racks in the worktable
- Checking the fraction input tubes

## Setting fraction collection parameters

You must configure the FractionLynx software to collect fractions before you begin processing samples. To edit a fraction file, select **Collection Parameters > Fraction File Editor** from the FractionLynx Editor. Refer to the *FractionLynx User's Guide* for additional information.

### Opening the FractionLynx editor

The FractionLynx Editor must be open to collect fractions. To open the FractionLynx Editor, select **FractionLynx > FractionLynx Editor** from the MassLynx Main window (see the [figure “MassLynx main window” on page 2-19](#)).

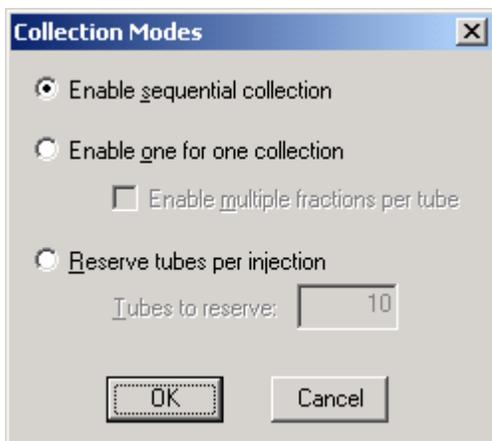
### Enabling fraction collection

To enable fraction collection, select **Fraction System > Enable Fraction Collection** from the FractionLynx Editor. The Enable Fraction Collection dialog box (see the [figure “Enabling fraction collection” on page 2-21](#)) appears. Select **Enable Fraction Collection** and click **OK** to close the dialog box.

## Setting collection mode

To set the collection method, select **Collection Parameters > Collection Modes** from the FractionLynx Editor. The Collection Modes dialog box appears.

### Setting the collection mode



### Enable sequential collection

Specifies that the collection vessels are used sequentially according to the parameters set in the Rack Generator window (see the [figure “Rack generator window”](#) on page 2-39).

### Enable one for one collection

Matches the plate position of the sample injection plate to the fraction collection vessel plate position. For example, it injects a sample from position 1 on the inlet plate and collects the fraction into position 1 on the fraction collection plate. If there is no sample identified for a position on the injection plate, no fraction is collected for that position on the fraction collection plate.

**Tip:** Only one fraction is collected for each sample and the volume of the fraction must fit into one fraction collection vessel.

Fraction collection using one for one collection plate mapping operates best with sample injection plates and collection plates containing the same number of positions. All sample injection positions desired must be run in a single sample batch.

**Tip:** For subsequent sample batches, the collection plate is considered full even if only one or two positions are used for fraction collection.

When a single collection plate contains fewer positions than the injection plate additional collection plates are mapped to the sample batch.

### **Reserve tubes per injection**

Allows a specified number of collection vessels to be designated for an injection. The collection vessels will be reserved for an injection whether or not fractions are collected into them. When selected, enter the number of tubes to reserve in the Tubes to reserve field. This option allows you to skip collection vessels between sample injections and to begin collecting fractions from injections at the beginning of rows or columns in the plate.

### **Using manual control**

To move the fraction dispense tube to any position on the worktable, select **Collector Setup > Manual Control** from the FractionLynx Editor. The Manual Control dialog box appears.

## Using manual control

The screenshot shows a software window titled "Manual Control" with a standard Windows-style title bar (minimize, maximize, close buttons). The interface is organized into several sections:

- Collector Number:** A dropdown menu currently showing "1".
- Move In Plate:** A sub-section containing a "Plate Number:" dropdown menu showing "1", a text input field containing "0", and a "Move" button.
- Move To Vessel:** A sub-section containing a text input field containing "0" and a "Move" button.
- Move XYZ (1/10 mms):** A sub-section containing three text input fields for X, Y, and Z, each containing "0", and a "Move" button.
- Fixed Positions:** A sub-section containing two buttons: "Move Home" and "Rinse Station".
- Valve:** A section at the bottom containing two radio buttons: "Divert to Vessel" (which is selected) and "Divert to Drain".

Ensure that the correct fraction collector appears in the Collector Number field.

### Move in plate

The Move In Plate option moves the fraction dispense tube to a selected vessel within the specified plate. Remember that plates are numbered from left to right on the worktable. Select the plate from the Plate Number list. Enter the vessel number in the box below Plate Number, then click to move the fraction dispense tube to the selected position.

### Move to vessel

When The Move To Vessel option is selected all of the vessels in the bed are numbered sequentially. If the bed layout has more than one rack, the numbers continue from the previous rack.

**Tip:** If an invalid vessel location is entered in either the Move In Plate or Move to Vessel area an error message appears and the fraction dispense tube moves to the closest vessel available.

### Move to XYZ (1/10 mms)

Enter the x-, y-, and z-coordinates, then click **Move** to move the fraction dispense tube to the selected position.

### Fixed positions

Click either **Move Home** or **Rinse Station** to move the fraction dispense tube to the rinse station. For the 2757 Sample Manager, the Home and rinse station locations are the same.

### Valve

Click **Divert to Vessel** or **Divert to Drain** to change the position of the fraction collect valve.

**Tip:** The Manual Control dialog box must be closed before processing samples.

## Resetting beds

Resetting the bed sets all of the collection beds to empty making the vessels available for fraction collection. The FractionLynx software stores the last fraction collection position and will resume at the next available vessel unless the status of a bed is reset.

To reset the bed before you begin processing samples, select **Reset Beds > Reset All Beds** from the FractionLynx Editor.

## Setting racks in the worktable

When you place fraction collection racks in the worktable, put the fraction collection rack containing the taller test tubes to the right of any fraction collection rack with shorter test tubes (see the [figure “Positioning fraction collection racks with different test tube heights in the worktable” on page 2-29](#)). For suitable fraction collection racks, see [“Selecting a rack” on page 2-28](#).

## Checking the fraction collection tube

The 2757 Sample Manager is equipped with a 0.020-inch ID fraction collection tube (see the [figure “Fraction collection mechanism” on page 1-7](#)), marked T9, for typical system operation. You can install a 0.010-inch ID fraction collection tube, marked T27, to optimize fraction collection for a low flow rate chromatography system. If you operate your chromatography system at a high flow rate, you can install a 0.040-inch ID fraction collection tube, marked T28. The Startup Kit contains both optional fraction collection tubes. Refer to the [table titled “Maximum flow rates for the fraction collection tubes” on page 2-11](#) for the maximum flow rate for each fraction collection tube.



# 4 Maintaining the 2757 Sample Manager

This chapter describes routine maintenance procedures you can perform to ensure the Waters 2757 Sample Manager consistently provides accurate and precise results. You also perform these procedures when you determine that a specific component in the 2757 Sample Manager has a problem. For information about isolating problems in the instrument, see [Chapter 5](#).

## Contents

| Topic  | Page |
|--|------|
| <a href="#">Maintenance considerations</a>                     | 4-2  |
| <a href="#">Cleaning the instrument</a>                        | 4-4  |
| <a href="#">Cleaning and lubricating the X/Y/Z robotic arm</a> | 4-4  |
| <a href="#">Replacing tubing</a>                               | 4-6  |
| <a href="#">Replacing fuses</a>                                | 4-8  |

## Maintenance considerations

---

Regularly performing maintenance helps to ensure accuracy and precision of the 2757 Sample Manager. The table below suggests intervals for the maintenance procedures to ensure uninterrupted operation. If you use the system heavily (for example, nights and weekends), or if you use aggressive solvents, you may need to perform the maintenance procedures more frequently.

### Suggested maintenance intervals

| Procedure                    | Interval                       | Reference  |
|------------------------------|--------------------------------|--|
| Maintain the X/Y/Z mechanism | Every 3 to 6 months, as needed | See “Cleaning and lubricating the X/Y/Z robotic arm” on page 4-4 |
| Replace the tubing           | As needed                      | See “Replacing tubing” on page 4-6                               |
| Replace fuses                | As needed                      | See “Replacing fuses” on page 4-8                                |

When you perform maintenance procedures on your 2757 Sample Manager, keep the following safety considerations in mind.



#### **Warning:**

- To avoid possible injury or system damage due to electrostatic discharge, do not touch integrated circuit chips or other components.
- To avoid possible electric shock, always turn off the instrument and unplug the power cord before performing the maintenance procedures in this chapter. Do not remove the back cover because it does not access any customer-serviceable parts.



**Warning:**

- To prevent injury, always observe safe laboratory practices when you handle solvents, change tubing, or operate the 2757 Sample Manager. Know the physical and chemical properties of the solvents you use. Refer to the Safety Material Data Sheets for the solvents in use.
- To avoid possible injury, always keep your hands outside the worktable and do not lean over the instrument during operation.
- Do not operate without proper safety shields. Safety shields must be installed for safe operation.



**Warning:** To avoid possible injury during 2757 Sample Manager operation, always keep your hands clear of the fraction dispense tube.



**Caution:** To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the 2757 Sample Manager. Once power is turned off, wait approximately 10 seconds before you disconnect an assembly.

## General operating procedures

To keep your 2757 Sample Manager running smoothly, follow the operating procedures and guidelines in [Chapter 3](#). Keep the fraction collection mechanism and the worktable clean for best results.

## Spare parts

See [Appendix C, Spare Parts and Options](#), for information. Parts not included in [Appendix C](#) are recommended for replacement by Waters service personnel.

## Contacting Waters technical service

If you encounter any problems replacing parts in the 2757 Sample Manager, contact Waters technical service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your Waters subsidiary or Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

## Cleaning the instrument

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Clean the fraction collection mechanism daily and the worktable components weekly for best results.



**Warning:** To avoid possible injury, ensure that the 2757 Sample Manager is powered off during cleaning.

### To clean the worktable:

1. Clean the shields and surfaces of the worktable with a lint-free cloth dampened with isopropyl alcohol.
2. If the fraction racks are dirty, clean them with a lint-free cloth dampened with isopropyl alcohol.

## Cleaning and lubricating the X/Y/Z robotic arm

---

Clean the X/Y/Z robotic arm every 3 to 6 months. If the instrument is operated in a dusty or humid environment, clean the X/Y/Z mechanism every 3 months.



**Caution:** To avoid serious damage to the instrument, do not clean the X/Y/Z axis guide rails or z-drive shaft with alcohol or any solvent. Use only isopropyl alcohol and a lint-free cloth to clean other parts of the 2757 Sample Manager. Other cleaning agents may affect the performance of the instrument.

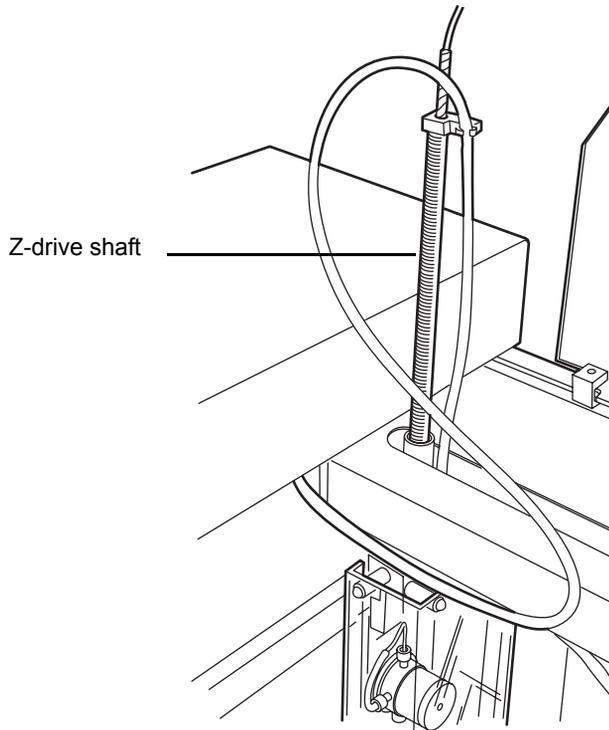
## Required materials

- Dry, lint-free cloth
- Lubricant

### To clean the X/Y/Z robotic arm:

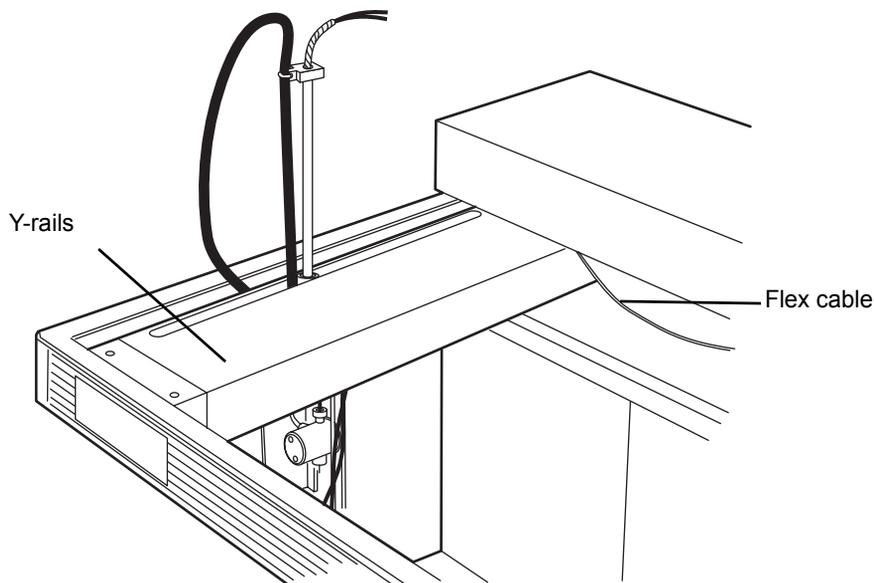
1. Power off the 2757 Sample Manager.
2. Clean the z-drive shaft with a dry, lint-free cloth.

## Cleaning the Z-drive shaft



3. Wipe the Y-arm using a lint-free cloth dampened with isopropyl alcohol to remove any residual dust.

## Cleaning the Y- and X-rails



4. Wipe the inside of the flex cable channel using a lint-free cloth dampened with isopropyl alcohol.



**Caution:** When cleaning the square shaft pinion, ensure that no alcohol enters the z-bearing or is wiped on the y-axis guide rails.

5. Wipe the square shaft pinion located underneath the arm using a lint-free cloth dampened with isopropyl alcohol.

## Replacing tubing

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All tubing must be kept clean and free of crimps. Tubing that is dirty, blocked, or crimped can result in poor accuracy and precision, loss of air gap, or syringe stalls. Replace the tubing if necessary. Frequency of replacement depends on duty cycle, reagents, and maintenance. The replacement part numbers for tubing are listed in [Appendix C, Spare Parts and Options](#).

## Fraction collection tube

The fraction collection tube connects the detector to the fraction dispense valve (see the [figure “Fraction collection mechanism” on page 1-7](#)). The standard 2757 Sample Manager is equipped with a 10-foot long fraction collection tube (T9) with a 0.020-inch ID. For optimum performance at low and high flow rates, optional fraction collection tubes are available:

- T27 for low flow rates, 0.010-in ID
- T28 for high flow rates, 0.040-in ID

Refer to the [table titled “Maximum flow rates for the fraction collection tubes” on page 2-11](#) for maximum flow rate for each fraction collection tube.

## Required material

Replacement fraction collection tube ([Appendix C, Spare Parts and Options](#))

### To replace the fraction collection tube:

1. Disconnect the fraction collection tube from the left side of the fraction dispense valve.
2. Disconnect the other end of the fraction collection tube from the detector.
3. Slide the tube out of the swiveling tube guide and fraction dispense apparatus.
4. Feed the new fraction collection tube through the swiveling tube guide and fraction dispense apparatus.
5. Connect the new fraction collection tube to the left side of the fraction dispense valve.
6. Connect the other end of the new fraction collection tube to the detector of C/FO.

## Replacing fuses

---

The 2757 Sample Manager uses two 5.0-A main fuses (see [Appendix B, Specifications](#)). The power supply automatically switches configuration for your power source (110/220 Vac operation). To prevent additional instrument downtime, replace both main fuses whenever one fuse fails.



**Warning:** To protect against fire hazard, replace fuses with the appropriate type and rating.

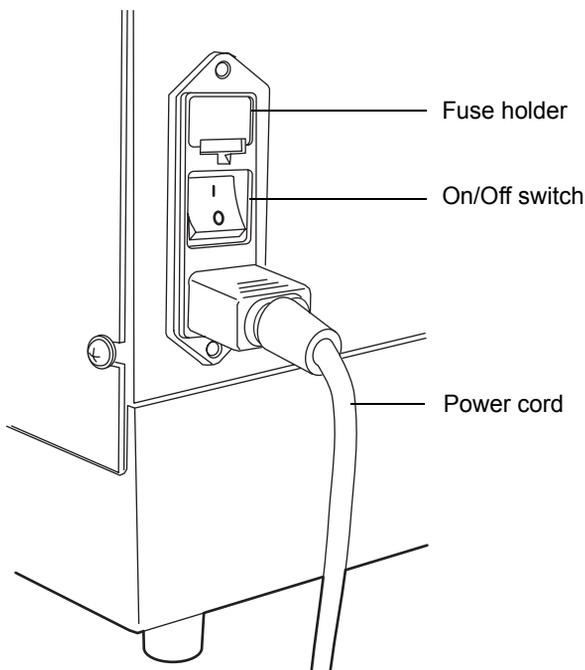
### Required material

Replacement ac fuse, 5.0 A (part number 700001394)

#### To replace a fuse:

1. Power off the 2757 Sample Manager.
2. Remove the fuse holder from the left side panel.

#### Replacing a fuse



3. Remove the fuses from the fuse holder.
4. Install two new fuses with the appropriate type and rating.
5. Install the fuse holder.
6. Power on the instrument.
7. If the fuse fails again in a short period of time, suspect another problem. See “Contacting Waters Technical Service” in [“Maintenance considerations”](#) on page 4-2.



# 5 Troubleshooting

This chapter describes how to find the source of and fix problems you may encounter with the 2757 Sample Manager. Information on troubleshooting software problems is in the following documents:

- *MassLynx Software Release Notes*
- *MassLynx User's Guide*
- *MassLynx Guide to Data Acquisition*
- *MassLynx Interfacing Guide*
- *MassLynx Guide to Inlet Control*
- *FractionLynx User's Guide*

## Contents

| Topic   | Page |
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| <a href="#">Spare parts</a>                         | 5-2  |
| <a href="#">Contacting Waters technical service</a> | 5-2  |
| <a href="#">Safety and handling</a>                 | 5-3  |
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| <a href="#">Fluidics</a>                            | 5-6  |
| <a href="#">Troubleshooting hardware problems</a>   | 5-7  |

## Proper operating procedures

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To keep your 2757 Sample Manager running optimally, follow the operating procedures and guidelines in [Chapter 3](#). Also, perform the maintenance procedures in [Chapter 4](#).

Check the 2757 Sample Manager periodically to determine that it is operating within acceptable parameters. Run control samples on a daily basis when running assays.

To troubleshoot problems with the instrument, refer to:

- See the [table titled “Error messages” on page 5-4](#) for information about error messages that have error codes
- See the [table titled “Troubleshooting hardware problems” on page 5-7](#) for information about hardware problems

## Spare parts

---

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

## Contacting Waters technical service

---

If you encounter any problems replacing parts in the 2757 Sample Manager, contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

## Safety and handling

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When you troubleshoot the 2757 Sample Manager, keep the following safety considerations in mind.



**Warning:**

- To avoid possible electrical shock, do not remove the back cover because it does not access any customer-serviceable parts.
- To avoid possible injury or system damage due to electrostatic discharge, do not touch integrated circuit chips or other components.



**Warning:** To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the 2757 Sample Manager. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.



**Warning:** To avoid possible injury, always keep your hands outside the worktable and do not lean over the instrument during operation.



**Caution:** To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the 2757 Sample Manager. Once power is turned off, wait approximately 10 seconds before you disconnect an assembly.

## Error messages

---

If an error occurs, one of the following messages appears. Use the table to find the error message with the possible cause and corrective action that you can take. Some error messages display the system component before the text of the message to indicate the source of the error.

### Error messages

| Error message        | Cause   | Corrective action   |
|----------------------|---|---|
| Initialization Error | Cannot initialize because robotic arm movement is obstructed. | Check the robotic arm movement mechanism for obstructions. Ensure that the shipping straps (see the <a href="#">figure “Unlocking the X/Y/Z robotic arm” on page 2-7</a> ) were removed from the robotic arm at installation. |
|                      | Dirt on X- and Y-rails.                                       | Clean rails (see <a href="#">“Cleaning and lubricating the X/Y/Z robotic arm” on page 4-4</a> ).  |
|                      | Fuse blown.   | Replace fuse (see <a href="#">“Replacing fuses” on page 4-8</a> ).  |
|                      | RS-232 cable loose or disconnected                            | Firmly connect cable.   |
|                      | No power to the 2757 Sample Manager                           | Power on the device.  |
|                      | Wrong communication port                                      | Set the correct COM port.   |
| Invalid Operand      | Values out of range.  | Check the coordinate values of the rinse station and each plate in the current bed layout (see <a href="#">“Verifying the rinse station location” on page 2-25</a> ).   |
|                      |   | Restart the software.   |

## Error messages (Continued)

| Error message                           | Cause   | Corrective action   |
|---|---|---|
| Device Not Implemented                  | Loose or damaged cables.                                    | Check the power and power cord.   |
|   |   | Check communications.   |
|   |   | Check cables and connections.   |
| Time-out Error                          | Loose or damaged communications cable.                      | Check communications.   |
|   | Device powered off.   | Check the instrument. Power on and reinitialize, by selecting <b>Fraction System &gt; Reset Communications</b> .                |
| Communications Timeout                  | Communication cable damaged or loose.                       | Check cables and connections.   |
|   | Device powered off.   | Check the instrument. Power on, if necessary, and reinitialize, by selecting <b>Fraction System &gt; Reset Communications</b> . |
| Not Initialized                         | Lost communication or device powered off.                   | Power on and reinitialize the device by selecting <b>Fraction System &gt; Reset Communications</b> .                            |
|   |   | Check RS-232 cable.   |
| No Acknowledgement Received from Device | Communication error with the device.                        | Reinitialize the device by selecting <b>Fraction System &gt; Reset Communications</b> .   |
|   | Device powered off.   | Check the instrument. Power on, if necessary, and reinitialize, by selecting <b>Fraction System &gt; Reset Communications</b> . |
| Z-axis Value Out of Range               | Entered invalid z-coordinate on the Position Adjust screen. | Enter a z-coordinate value less than the current value.   |

## Error messages (Continued)

| Error message  | Cause  | Corrective action  |
|--|--|--|
| Step Loss Detected on X-axis or Step Loss Detected on Y-axis | Movement of the arm obstructed.                    | Reposition the tubes and cables to allow arm to move freely. Clear any obstructions.   |
|  | Dirt accumulated on rails.                         | Initialize the device by selecting <b>Fraction System &gt; Reset Communications</b> .<br>Clean the rails (see the <a href="#">figure “Cleaning and lubricating the X/Y/Z robotic arm”</a> on page 4-4).      |
| Step Loss Detected on Z-axis                                 | Movement of the fraction dispense tube obstructed. | Check the movement mechanism for obstructions.<br>Check coordinates of the rinse station and each plate in the current bed layout (see <a href="#">“Verifying the rinse station location”</a> on page 2-25). |
| Unable to Open Bed Information                               | File containing bed definitions deleted.           | Recreate bed definitions.  |
| Unable to Open Plate Information                             | File containing plate definitions deleted.         | Recreate plate definitions.  |

## Fluidics

---

This section contains information on the fluidic components. See [Appendix D](#), for specific information on solvents.

### Liquid handling rules

When implementing programs or methods, operators should be familiar with general rules of liquid handling. Treat all samples, standards, and reference controls alike.

## Quality control

Routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of range may not be valid. Do not report this data until you ensure that chromatographic system performance is acceptable.

## Troubleshooting hardware problems

---

This section provides information for troubleshooting the 2757 Sample Manager. If the suggested solutions do not solve the problem, see [“Contacting Waters technical service” on page 5-2](#).

### Troubleshooting hardware problems

| Symptom                | Possible cause  | Corrective action   |
|------------------------|---|---|
| Fluid found on racks   | The tubing is loose.  | Tighten the tubing fittings.  |
| Fluid in the drip tray | The tubing has leaks, blockages, crimps, or air bubbles.                                | Tighten or straighten the fittings. If the tubing still has leaks, blockages, crimps, or air bubbles, replace the tubing (see <a href="#">“Replacing tubing” on page 4-6</a> ). |
|                        | The wash station is blocked.  | Clean the wash station and check the fittings.  |
|                        | The wash station tubing is damaged, blocked, loose, or leaking.                         | Tighten or replace the tubing.  |
|                        | The tubing to the waste container has a trap in the tubing.                             | Ensure that fluid flows freely into the waste container by vertically straightening out the tubing.   |
|                        | The end of the waste fluid tubing is submerged in fluid or foam in the waste container. | Ensure that the end of the tubing is not submerged in fluid or foam.  |
|                        | Racks are wet from earlier cleaning or spills.  | Clean and dry the racks thoroughly.   |

## Troubleshooting hardware problems (Continued)

| Symptom                            | Possible cause  | Corrective action   |
|------------------------------------|---|---|
| Fluid in the drip tray (continued) | Wrong plate selected in bed layout                                  | Select correct plates (see the <a href="#">figure “Selecting the bed layout for fraction collection” on page 2-23</a> ).                                    |
| Broken vials                       | Sample containers or racks are not installed correctly.             | Ensure that sample containers and racks are installed correctly.  |
|                                    | The fraction dispense tube is not calibrated to the correct height. | Check coordinates of the rinse station and each plate in the current bed layout (see <a href="#">“Verifying the rinse station location” on page 2-25</a> ). |
| Cannot initialize                  | RS-232 connector to MassLynx workstation is loose.                  | Tighten connection (see <a href="#">“Installing the communications cable” on page 2-15</a> ).   |
|                                    | Communication port setting is incorrect.                            | Select proper communications port.  |
|                                    | The fuse is blown.  | Replace the main fuse (see <a href="#">“Replacing fuses” on page 4-8</a> ).   |
|                                    | Device is not turned on.  | Power on the 2757 Sample Manager.   |
|                                    | The power supply has a problem.                                     | Contact Waters Technical Service (see <a href="#">“Contacting Waters technical service” on page 5-2</a> ).  |
|                                    | The cables are not securely plugged in.                             | Securely fasten all cables and the power cord.  |
| Intermittent run failures          | The cables are not securely plugged in.                             | Securely fasten all cables and the power cord.  |
|                                    | A cable is damaged.   | Replace cables one at a time until the problem is solved.   |
|                                    | RS-232 connector to MassLynx workstation is loose.                  | Tighten connection (see <a href="#">“Installing the communications cable” on page 2-15</a> ).   |

### Troubleshooting hardware problems (Continued)

| Symptom                | Possible cause                             | Corrective action  |
|------------------------|--|--|
| Inoperative instrument | The instrument power is off.               | Power on the 2757 Sample Manager.  |
|                        | The power cord is unplugged at either end. | Check the power cord at the instrument and at the outlet.                    |
|                        | A main fuse is blown.                      | Replace the main fuses (see <a href="#">“Replacing fuses” on page 4-8</a> ). |
|                        | Power to the outlet is off.                | Ensure that the power to the outlet is on.                                   |



# A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

## Contents

| Topic   | Page                 |
|---|----------------------|
| <a href="#">Warning symbols</a>                               | <a href="#">A-2</a>  |
| <a href="#">Caution symbol</a>                                | <a href="#">A-5</a>  |
| <a href="#">Warnings that apply to all Waters instruments</a> | <a href="#">A-6</a>  |
| <a href="#">Electrical and handling symbols</a>               | <a href="#">A-12</a> |

## Warning symbols

---

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

### Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific 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 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 eye injury.)

## Specific warnings

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

### Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.



**Warning:** Pressurized nonmetallic, or polymer, tubing can burst.

Observe these precautions when working around such tubing:

- 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 incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

## Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.



**Warning:** Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 690 kPa (6.9 bar, 100 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the LC system so that the LC solvent flow stops if the nitrogen supply fails.

## Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.



**Warning:** To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.

This warning applies to certain instruments when they are in Operate mode.



**Warning:** High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid non-lethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

## Biohazard warning

This warning applies to Waters instruments that can be used to process material that might contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.



**Warning:** Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

## Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.



**Warning:** Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*.

## Caution symbol

---

The caution symbol signifies that an instrument's use or misuse can damage the instrument or compromise a sample's integrity. The following symbol and its associated statement are typical of the kind that alert you to the risk of damaging the instrument or sample.



**Caution:** To avoid damage, do not use abrasives or solvents to clean the instrument's case.

## Warnings that apply to all Waters instruments

---

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:** 未经有关法规认证部门明确允许对本设备进行的改变或改装,可能会使使用者丧失操作该设备的合法性。

**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.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez 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。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 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砜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 디메틸설폭시드(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。



**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.



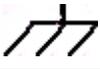
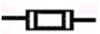
**警告:** 火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

# Electrical and handling symbols

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## Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

|   |  |
|---|--|
|    | Electrical power on                                |
|    | Electrical power off                               |
|    | Standby  |
|    | Direct current                                     |
|    | Alternating current                                |
|   | Protective conductor terminal                      |
|  | Frame, or chassis, terminal                        |
|  | Fuse   |
|  | Recycle symbol: Do not dispose in municipal waste. |

## Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

|   |               |
|---|---------------|
|  | Keep upright! |
|  | Keep dry!     |
|  | Fragile!      |
|  | Use no hooks! |



# B Specifications

This appendix lists the following specifications:

- Physical specifications
- Environmental specifications
- Electrical specifications
- Operational specifications

**Tip:** The performance characteristics stated in the specifications were not established by any in-vitro diagnostic assays. It is the sole responsibility of each laboratory to verify that the system works for its applications.

## Physical specifications

| Item   | Specification   |
|--|---|
| Footprint with required clearance:<br>Height<br>Depth<br>Width | 30 in. (76.2 cm); 33 in. (83.8 cm) with optional fume hood<br>24.5 in. (62.2 cm)<br>34 in. (86.4 cm)              |
| Weight   | 120 pounds (59.4 kg)  |
| Worktable<br>(x × y × z)                                       | 25.4 in. × 11.8 in. × 6.7 in. (64.5 cm × 30.0 cm × 17.0 cm)   |
| Wetted surface materials                                       | 316L stainless steel, Tefzel <sup>®</sup> (ETFE), Teflon <sup>®</sup> (FEP and PTFE), polyetheretherketone (PEEK) |
| Ventilation  | At least 1 in. (2.5 cm) from a wall or obstructing surface  |
| Safety   | UL, C-UL, CE, IEC 1010-1  |

## Environmental specifications

| Item                  | Specification              |
|-----------------------|----------------------------|
| Operating temperature | 15 to 40 °C (59 to 104 °F) |

## Environmental specifications (Continued)

| Item                  | Specification   |
|-----------------------|---|
| Operating environment | Indoor use  |
| Relative humidity     | Maximum relative humidity 85% for temperatures up to 31 °C, decreasing linearly to 50% relative humidity at 40 °C |
| Storage temperature   | 0 to 50 °C (32 to 122 °F)   |
| Altitude              | Up to 2,000 meters (6562 feet)  |

## Electrical specifications

| Item                  | Specification   |
|-----------------------|---|
| Power supply input    | Auto-selecting 110/230 Vac $\pm 10\%$ , 47 to 63 Hz, maximum 300 watts. Requires earth-grounded power source. |
| Fuses                 | Two 5-A fuses   |
| Power supply output   | Generates 24 V. All services internal to the instrument operate on 24 V.                                      |
| Installation category | Overvoltage Category II   |
| Input voltages        | Auto-selecting 110/230 Vac $\pm 10\%$   |
| Frequency             | 47 to 63 Hz   |
| Maximum power output  | 250 watts   |

## Operational specifications

| Item         | Specifications |        |        |
|--------------|----------------|--------|--------|
|              | X-axis         | Y-axis | Z-axis |
| Travel range |                |        |        |
| mm           | 645            | 300    | 170    |
| in.          | 25.40          | 11.81  | 6.69   |
| Resolution   |                |        |        |

### Operational specifications (Continued)

| Item  | Specifications |        |        |
|---|----------------|--------|--------|
|   | X-axis         | Y-axis | Z-axis |
| mm  | 0.2234         | 0.0142 | 0.0982 |
| in.   | 0.009          | 0.006  | 0.004  |
| Accuracy over travel <sup>a</sup>             |                |        |        |
| mm  | ±0.20          | ±0.14  | ±0.14  |
| in.   | ±0.008         | ±0.006 | ±0.006 |
| Max Acceleration<br>(steps/sec <sup>2</sup> ) | 5000           | 10,000 | 30,000 |
| Max Velocity (steps/sec <sup>2</sup> )        | 3300           | 3300   | 4400   |

a. Accuracy and precision were determined within run, and coordinates were measured independently.



# C Spare Parts and Options

The contents of the Startup Kit are listed on a packing slip included with the shipment of your 2757 Sample Manager.

**Tip:** Parts not included in the [table titled “Spare parts” on page C-2](#) must be replaced by Waters service personnel.

## Contents

| Topic                       | Page                |
|-----------------------------|---------------------|
| <a href="#">Spare parts</a> | <a href="#">C-2</a> |
| <a href="#">Options</a>     | <a href="#">C-3</a> |

## Spare parts

---

For recommended spare parts, see the table below.

### Spare parts

| Item  | Part number |
|---|-------------|
| <b>Racks</b>                                |             |
| 96-well microplate holder                   | 205000105   |
| 13-mm tube holder                           | 205000108   |
| 2-mL vial holder                            | 205000107   |
| 4-mL vial holder                            | 205000106   |
| Random access 2-mL vial holder kit          | 205000109   |
| Open access 4-mL vial holder kit            | 205000112   |
| Deep well plate fraction collector rack kit | 205000134   |
| 13-mm fraction collector rack kit           | 205000116   |
| 16-mm fraction collector rack kit           | 205000117   |
| 18-mm fraction collector rack kit           | 205000115   |
| 25-mm fraction collector rack kit           | 205000118   |
| 28-mm fraction collector rack kit           | 205000119   |
| <b>Miscellaneous parts</b>                  |             |
| Fraction collection tube, T9, 0.020 in. ID  | 700001682   |
| Fraction collection tube, T27, 0.010 in. ID | 700001656   |
| Fraction collection tube, T28, 0.040 in. ID | 700001657   |
| Wash station                                | 700001399   |
| Feet (4 per package)                        | 700001416   |
| Drip tray                                   | 700001417   |
| Flex ribbon cable                           | 700001397   |
| <b>Tools</b>                                |             |
| Flat-blade screwdriver                      | 700001432   |
| Metric Allen wrench set                     | 700001431   |
| Spare screw kit                             | 700001433   |

# Options

---

The following table lists the options available with the 2757 Sample Manager.

## Options

| Item               | Part number    |
|--------------------|----------------|
| Fume hood          | 205000125      |
| Leak sensor module | Contact Waters |



# D Solvent Considerations.

 **Warning:** To avoid chemical hazards, always observe safe laboratory practices when operating your system.

## Contents

| Topic                 | Page |
|-----------------------|------|
| Introduction          | D-2  |
| Solvent compatibility | D-3  |
| Solvent miscibility   | D-6  |
| Buffered solvents     | D-8  |
| Solvent stabilizers   | D-9  |
| Solvent viscosity     | D-9  |
| Solvent degassing     | D-9  |
| Wavelength selection  | D-11 |

# Introduction

---

## 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 filters with particulate matter

## Solvent quality

Use HPLC-grade solvents to ensure the best possible results. Filter solvents 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.

## Preparation checklist

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

- Filter buffered solvents with a 0.45- $\mu\text{m}$  filter.
- Degas solvents.
- Keep solvents in a place free from drafts and shock.

## Water

Use water only from a high-quality 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.

## Buffers

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

## Tetrahydrofuran (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.

## Solvent compatibility

---

The 2757 Sample Manager has the following wetted materials consisting of 316L stainless steel, Tefzel (ETFE), Teflon (FEP and PTFE), polyetheretherketone (PEEK). These materials are compatible with nearly all chromatographic solvents and modifiers. This section lists all the solvents that have and have not been approved for used with the 2757 Sample Manager. It includes cautionary notes, including limits of concentrations for modifiers.

### Solvents to avoid

You can use all chromatographic solvents with the 2757 Sample Manager. However, long-term *static* exposure to halide salts (for example, fluoride, bromide, chloride, and iodide) will cause pitting and corrosion of stainless steel parts. When using these salts, flush your system thoroughly with water if the pump will be idle for more than two days. 100% THF and acetonitrile (ACN) concentrations result in de-rated high pressure limit performance for PEEK tubing and should be taken into consideration whenever the original system flow path is modified.

### Solvents to use

Materials of construction used in the 2757 Sample Manager are nonreactive with most acids, bases, salts, and organic solvents.

The solvents listed in the tables below include many solvents not expected to be in common use with the 2757 Sample Manager.

**Tip:** The most commonly used items are preceded by two asterisks (\*\*).

Mobile phase modifiers including salt buffers up to 100 mm, acids up to 100 mm, and bases up to 1 M are listed unless otherwise noted. Organic solvents can be used in concentrations up to 100%.

Information on the use of a specific solvent or concentration that is not listed in this manual can be obtained by contacting Waters.

#### Aqueous buffers for use with the 2757 Sample Manager

| Aqueous buffers                               |   |                       |
|---|---|-----------------------|
| Acetate                                       | K <sub>2</sub> SO <sub>4</sub>                | NaHCO <sub>3</sub>    |
| Al <sub>2</sub> SO <sub>4</sub>               | K <sub>3</sub> Fe(CN) <sub>6</sub>            | NaHSO <sub>4</sub>    |
| **Ammonium acetate                            | K <sub>4</sub> Fe(CN) <sub>6</sub>            | NaNO <sub>3</sub>     |
| **Ammonium bicarbonate                        | KBr   | NaOCl                 |
| **Ammonium formate                            | KCl   | NH <sub>4</sub> Cl    |
| CaCl <sub>2</sub>                             | KHCO <sub>3</sub>                             | Perfluorobutyric acid |
| Ca(OCl) <sub>2</sub>                          | KNO <sub>3</sub>                              | **Phosphate           |
| **Citric acid                                 | LiClO <sub>4</sub>                            | **Sodium acetate      |
| **EDTA  | Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> | **Sodium citrate      |
| H <sub>2</sub> O <sub>2</sub> up to 10%       | Na <sub>2</sub> CO <sub>3</sub>               | Tartrate              |
| HIBA  | Na <sub>2</sub> SO <sub>4</sub>               | Trilithium citrate    |
| K <sub>2</sub> CO <sub>3</sub>                | NaCl  | Tris                  |
| K <sub>2</sub> Cr <sub>2</sub> O <sub>3</sub> | NaH <sub>2</sub> BO <sub>3</sub>              |                       |

#### Acids for use with the 2757 Sample Manager

| Acids                  |                                       |   |
|------------------------|---------------------------------------|---|
| **Acetic acid, glacial | Hydrochloric acid <3 mm               | Phosphoric acid                         |
| Chromic acid           | Nitric acid, up to 37.5% (6N cleaner) | Pyridine-2,6-dicarboxylic acid          |
| **Formic acid          | Oxalic acid                           | **Trifluoroacetic acid (TFA), up to 10% |
| Glyceric acid          | Perchloric acid                       |   |

## Bases for use with the 2757 Sample Manager

| Bases               |  |
|---------------------|--|
| Ba(OH) <sub>2</sub> | **NaOH, up to 10 M                         |
| KOH                 | **NH <sub>4</sub> OH, up to 3 M            |
| LiOH                | Tetramethylammonium hydroxide pentahydrate |

## Organic solvents for use with the 2757 Sample Manager

| Organic solvents |                      |                     |                         |
|------------------|----------------------|---------------------|-------------------------|
| 4-cyanophenol    | Carbon tetrachloride | Ethylene dichloride | Methyl ethyl ketone     |
| Acetone          | Chloroform           | Ethylene glycol     | Methylene chloride      |
| **Acetonitrile   | Cyclohexane          | Formaldehyde        | ** <i>n</i> -Propanol   |
| Amyl acetate     | Cyclohexanone        | Heptane             | Phenol                  |
| Benzaldehyde     | Dibutyl phthalate    | Hexane              | **Tetrahydrofuran (THF) |
| Benzene          | Dimethyl formamide   | Isooctane           | Toluene                 |
| Benzyl alcohol   | Ethanol              | Isopropanol         | Xylene                  |
| Butanol          | Ethyl acetate        | **Methanol          |                         |

## Solvent miscibility

Before you change solvents, see the table below to determine the miscibility of the solvents to be used, When you change solvents, be aware that:

- Changes involving two miscible solvents may be made directly. Changes involving two solvents that are not totally miscible (for example, chloroform and water) require an intermediate solvent (such as *n*-propanol).
- Temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of higher temperature on solvent solubility.
- Buffers dissolved in water may precipitate when mixed with organic solvents.

### Solvent miscibility

| Polarity index | Solvent            | Viscosity CP, 20 °C | Boiling point °C (@1 atm) | Miscibility number (M) | $\lambda$ Cutoff (nm) |
|----------------|--------------------|---------------------|---------------------------|------------------------|-----------------------|
| -0.3           | N-decane           | 0.92                | 174.1                     | 29                     | —                     |
| -0.4           | Iso-octane         | 0.50                | 99.2                      | 29                     | 210                   |
| 0.0            | N-hexane           | 0.313               | 68.7                      | 29                     | —                     |
| 0.0            | Cyclohexane        | 0.98                | 80.7                      | 28                     | 210                   |
| 1.7            | Butyl ether        | 0.70                | 142.2                     | 26                     | —                     |
| 1.8            | Triethylamine      | 0.38                | 89.5                      | 26                     | —                     |
| 2.2            | Isopropyl ether    | 0.33                | 68.3                      | —                      | 220                   |
| 2.3            | Toluene            | 0.59                | 100.6                     | 23                     | 285                   |
| 2.4            | <i>P</i> -xylene   | 0.70                | 138.0                     | 24                     | 290                   |
| 3.0            | Benzene            | 0.65                | 80.1                      | 21                     | 280                   |
| 3.3            | Benzyl ether       | 5.33                | 288.3                     | —                      | —                     |
| 3.4            | Methylene chloride | 0.44                | 39.8                      | 20                     | 245                   |
| 3.7            | Ethylene chloride  | 0.79                | 83.5                      | 20                     | —                     |
| 3.9            | Butanol            | 3.01                | 117.2                     | 15                     | —                     |
| 4.2            | Tetrahydrofuran    | 0.55                | 66.0                      | 17                     | 220                   |
| 4.3            | Ethyl acetate      | 0.47                | 77.1                      | 19                     | 260                   |

### Solvent miscibility (Continued)

| Polarity index | Solvent             | Viscosity CP, 20 °C | Boiling point °C (@1 atm) | Miscibility number (M) | $\lambda$ Cutoff (nm) |
|----------------|---------------------|---------------------|---------------------------|------------------------|-----------------------|
| 4.3            | 1-propanol          | 2.30                | 97.2                      | 15                     | 210                   |
| 4.3            | 2-propanol          | 2.35                | 117.7                     | 15                     | —                     |
| 4.4            | Methyl acetate      | 0.45                | 56.3                      | 15, 17                 | 260                   |
| 4.5            | Methyl ethyl ketone | 0.43                | 80.0                      | 17                     | 330                   |
| 4.5            | Cyclohexanone       | 2.24                | 155.7                     | 28                     | 210                   |
| 4.5            | Nitrobenzene        | 2.03                | 210.8                     | 14, 20                 | —                     |
| 4.6            | Benzonitrile        | 1.22                | 191.1                     | 15, 19                 | —                     |
| 4.8            | Dioxane             | 1.54                | 101.3                     | 17                     | 220                   |
| 5.2            | Ethanol             | 1.20                | 78.3                      | 14                     | 210                   |
| 5.3            | Pyridine            | 0.94                | 115.3                     | 16                     | 305                   |
| 5.3            | Nitroethane         | 0.68                | 114.0                     | —                      | —                     |
| 5.4            | Acetone             | 0.32                | 56.3                      | 15, 17                 | 330                   |
| 5.5            | Benzyl alcohol      | 5.80                | 205.5                     | 13                     | —                     |
| 5.7            | Methoxyethanol      | 1.72                | 124.6                     | 13                     | —                     |
| 6.2            | Acetonitrile        | 0.37                | 81.6                      | 11, 17                 | 190                   |
| 6.2            | Acetic acid         | 1.26                | 117.9                     | 14                     | —                     |
| 6.4            | Dimethylformamide   | 0.90                | 153.0                     | 12                     | —                     |
| 6.5            | Dimethylsulfoxide   | 2.24                | 189.0                     | 9                      | —                     |
| 6.6            | Methanol            | 0.60                | 64.7                      | 12                     | 210                   |
| 7.3            | Formamide           | 3.76                | 210.5                     | 3                      | —                     |
| 9.0            | Water               | 1.00                | 100.0                     | —                      | —                     |

### How to use miscibility numbers (M-numbers)

Use miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent (see the [table titled “Solvent miscibility” on page D-6](#)).

To predict the miscibility of two liquids, subtract the smaller M-number value from the larger M-number value.

- If the difference between the two M-numbers is 15 or less, the two liquids are miscible in all proportions at 15 °C.
- A difference of 16 indicates a critical solution temperature from 25 to 75 °C, with 50 °C as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible or their critical solution temperature is above 75 °C.

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents receive a dual M-number:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents.
- The second number applies to the opposite end of the scale. A large difference between these two numbers indicates a limited range of miscibility.

For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0 and 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

## Buffered solvents

---

When using a buffer, use a good quality reagent and filter it through a 0.45- $\mu$ m filter.

Do not leave the buffer stored in the system after use. Flush all fluidic pathways with HPLC-quality water before shutting down the system and leave distilled water in the system (flush with 90% HPLC-quality water:10% methanol for shutdowns scheduled to be more than one day). Flush using a minimum of 15 mL for sparge-equipped units, and a minimum of 45 mL for inline vacuum degasser-equipped units.

## Solvent stabilizers

---

Solvents containing stabilizers, for example, THF with butylated hydroxytoluene (BHT), should never be left to dry out in the system flow path. A dried system flow path, including the detector flow cell, will become contaminated with residual stabilizer requiring substantial cleaning to restore cleanliness to initial conditions.

## Solvent viscosity

---

Generally, viscosity is not important when you are operating with a single solvent or under low pressure. However, when you run a gradient, the viscosity changes that occur as the solvents are mixed in different proportions can result in pressure changes during the run. For example, a 1:1 mixture of water and methanol produces twice the pressure of either water or methanol alone.

If the extent to which the pressure changes affect the analysis is not known, monitor the pressure during the run.

## Solvent degassing

---

Mobile phase difficulties account for 70 percent or more of all liquid chromatographic problems. Using degassed solvents is important, especially at wavelengths below 220 nm. Degassing provides:

- Stable baselines and enhanced sensitivity
- Reproducible retention times for eluting peaks
- Reproducible injection volumes for quantitation
- Stable pump operation

This section presents information on the solubility of gases and solvent degassing methods.

## Gas solubility

The amount of gas dissolved in a given volume of liquid depends on:

- The chemical affinity of the gas for the liquid
- The temperature of the liquid
- The pressure applied to the liquid

Changes in the composition, temperature, or pressure of the mobile phase can lead to outgassing.

### Effects of intermolecular forces

Nonpolar gases ( $N_2$ ,  $O_2$ ,  $CO_2$ , and He) are more soluble in nonpolar solvents than in polar solvents. Generally, a gas is most soluble in a solvent whose intermolecular attractive forces are similar to those in the gas (“like dissolves like”).

### Effects of temperature

Temperature affects the solubility of gases. If the heat of solution is exothermic, the solubility of the gas decreases when you heat the solvent. If the heat of solution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in  $H_2O$  decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

### Effects of partial pressure

The mass of gas dissolved in a given volume of solvent is proportional to the partial pressure of the gas in the vapor phase of the solvent. If you decrease the partial pressure of the gas, the amount of that gas in solution also decreases.

## Solvent degassing methods

You can degas solvents by either of the following methods:

- Sparging with helium
- Vacuum degassing

## Sparging

Helium sparging gives stable baselines and better detection sensitivity than sonication, and prevents reabsorption of atmospheric gases. Use this method to retard oxidation when you are using THF or other peroxide-forming solvents.

## Vacuum degassing

The longer a solvent is exposed to a vacuum, the more dissolved gases are removed. Two factors affect the amount of time the solvent is exposed to the vacuum:

- Flow rate – At low flow rates, most of the dissolved gas is removed as the solvent passes through the vacuum chamber. At higher flow rates, lesser amounts of gas per unit volume of solvent are removed.
- Surface area of the degassing membrane – The length of the degassing membrane is fixed in each vacuum chamber. To increase the length of membrane, you can connect two or more vacuum chambers in series.

## Wavelength selection

---

The tables in this section provide UV cutoff values for:

- Common solvents
- Common mixed mobile phases
- Chromophores

## UV cutoffs for common solvents

The [table titled “UV cutoff wavelengths for common chromatographic solvents” on page D-11](#) shows the UV cutoff (the wavelength at which the absorbance of the solvent is equal to 1 AU) for some common chromatographic solvents. Operating at a wavelength near or below the cutoff increases baseline noise due to the absorbance of the solvent.

### UV cutoff wavelengths for common chromatographic solvents

| Solvent        | UV cutoff (nm) | Solvent         | UV cutoff (nm) |
|----------------|----------------|-----------------|----------------|
| 1-Nitropropane | 380            | Ethylene glycol | 210            |

## UV cutoff wavelengths for common chromatographic solvents (Continued)

| Solvent              | UV cutoff (nm) | Solvent                   | Uv cutoff (nm) |
|----------------------|----------------|---------------------------|----------------|
| 2-Butoxyethanol      | 220            | Isooctane                 | 215            |
| Acetone              | 330            | Isopropanol               | 205            |
| Acetonitrile         | 190            | Isopropyl chloride        | 225            |
| Amyl alcohol         | 210            | Isopropyl ether           | 220            |
| Amyl chloride        | 225            | Methanol                  | 205            |
| Benzene              | 280            | Methyl acetate            | 260            |
| Carbon disulfide     | 380            | Methyl ethyl ketone       | 330            |
| Carbon tetrachloride | 265            | Methyl isobutyl ketone    | 334            |
| Chloroform           | 245            | Methylene chloride        | 233            |
| Cyclohexane          | 200            | <i>n</i> -Pentane         | 190            |
| Cyclopentane         | 200            | <i>n</i> -Propanol        | 210            |
| Diethyl amine        | 275            | <i>n</i> -Propyl chloride | 225            |
| Dioxane              | 215            | Nitromethane              | 380            |
| Ethanol              | 210            | Petroleum ether           | 210            |
| Ethyl acetate        | 256            | Pyridine                  | 330            |
| Ethyl ether          | 220            | Tetrahydrofuran           | 230            |
| Ethyl sulfide        | 290            | Toluene                   | 285            |
| Ethylene dichloride  | 230            | Xylene                    | 290            |

## Mixed mobile phases

The [table titled “Wavelength cutoffs for different mobile phases” on page D-13](#) provides approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most commonly used. To use a different concentration, determine

approximate absorbance using Beer's Law, since absorbance is proportional to concentration.

### Wavelength cutoffs for different mobile phases

| Mobile phase  | UV cutoff (nm) | Mobile phase                                 | UV cutoff (nm) |
|---|----------------|--|----------------|
| Acetic acid, 1%   | 230            | Sodium chloride, 1 M                         | 207            |
| Ammonium acetate, 10 mM                                 | 205            | Sodium citrate, 10 mM                        | 225            |
| Ammonium bicarbonate, 10 mM                             | 190            | Sodium dodecyl sulfate                       | 190            |
| BRIJ 35, 0.1%   | 190            | Sodium formate, 10 mM                        | 200            |
| CHAPS, 0.1%   | 215            | Triethyl amine, 1%                           | 235            |
| Diammonium phosphate, 50 mM                             | 205            | Trifluoroacetic acid, 0.1%                   | 190            |
| EDTA, disodium, 1 mM                                    | 190            | TRIS HCl, 20 mM, pH 7.0, pH 8.0              | 202, 212       |
| HEPES, 10 mM, pH 7.6                                    | 225            | Triton-X™ 100, 0.1%                          | 240            |
| Hydrochloric acid, 0.1%                                 | 190            | Waters PIC® Reagent A, 1 vial/liter          | 200            |
| MES, 10 mM, pH 6.0                                      | 215            | Waters PIC Reagent B-6, 1 vial/liter         | 225            |
| Potassium phosphate, monobasic, 10 mM<br>dibasic, 10 mM | 190<br>190     | Waters PIC Reagent B-6, low UV, 1 vial/liter | 190            |
| Sodium acetate, 10 mM                                   | 205            | Waters PIC Reagent D-4, 1 vial/liter         | 190            |

### Refractive indices of common solvents

The table titled “Refractive indices for common chromatographic solvents” on page D-14 lists the refractive indices for some common chromatographic solvents. Use this table to verify that the solvent you intend to use for your

analysis has a refractive index (RI) significantly different from the RIs of the sample components.

### Refractive indices for common chromatographic solvents

| Solvent                      | RI     | Solvent                               | RI    |
|------------------------------|--------|---------------------------------------|-------|
| Fluoroalkanes                | 1.25   | Tetrahydrofuran (THF)                 | 1.408 |
| Hexafluoroisopropanol (HFIP) | 1.2752 | Amyl alcohol                          | 1.410 |
| Methanol                     | 1.329  | Diisobutylene                         | 1.411 |
| Water                        | 1.33   | <i>n</i> -Decane                      | 1.412 |
| Acetonitrile                 | 1.344  | Amyl chloride                         | 1.413 |
| Ethyl ether                  | 1.353  | Dioxane                               | 1.422 |
| <i>n</i> -Pentane            | 1.358  | Ethyl bromide                         | 1.424 |
| Acetone                      | 1.359  | Methylene chloride                    | 1.424 |
| Ethanol                      | 1.361  | Cyclohexane                           | 1.427 |
| Methyl acetate               | 1.362  | Ethylene glycol                       | 1.427 |
| Isopropyl ether              | 1.368  | <i>N,N</i> -Dimethyl Formamide (DMF)  | 1.428 |
| Ethyl acetate                | 1.370  | <i>N,N</i> -Dimethyl Acetamide (DMAC) | 1.438 |
| 1-Pentene                    | 1.371  | Ethyl sulfide                         | 1.442 |
| Acetic acid                  | 1.372  | Chloroform                            | 1.443 |
| Isopropyl chloride           | 1.378  | Ethylene dichloride                   | 1.445 |
| Isopropanol                  | 1.38   | Carbon tetrachloride                  | 1.466 |
| <i>n</i> -Propanol           | 1.38   | Dimethyl sulfoxide (DMSO)             | 1.477 |
| Methylethylketone            | 1.381  | Toluene                               | 1.496 |
| Diethyl amine                | 1.387  | Xylene                                | ~1.50 |
| <i>n</i> -Propyl chloride    | 1.389  | Benzene                               | 1.501 |
| Methylisobutylketone         | 1.394  | Pyridine                              | 1.510 |
| Nitromethane                 | 1.394  | Chlorobenzene                         | 1.525 |
| 1-Nitropropane               | 1.400  | <i>o</i> -Chlorophenol                | 1.547 |
| Isooctane                    | 1.404  | Aniline                               | 1.586 |
| Cyclopentane                 | 1.406  | Carbon disulfide                      | 1.626 |

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