

ACQUITY UPLC Fluorescence Detector

Getting Started Guide

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Waters contact information

Contacting medium	Information
Internet	The Waters Web site includes contact information for Waters locations worldwide. Visit www.waters.com and click Waters Division.
Telephone and fax	From the USA or Canada, phone 800 252-HPLC, or fax 508 872 1990. For other locations worldwide, phone and fax numbers appear in the Waters Web site.
Conventional mail	Waters Corporation 34 Maple Street Milford, MA 01757 USA

Safety considerations

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 Practices, and consult your organization's safety representative for guidance.

When you develop methods, follow the "Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory," *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol addresses good operating procedures and the techniques necessary to validate system and method performance.

Safety advisories

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

Operating this instrument

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

Applicable symbols

Symbol	Definition
	Confirms that a manufactured product complies with all applicable European Community directives
 ABN 49 065 444 751	Australia C-Tick EMC Compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements

Audience and purpose

This guide is intended for personnel who install, operate, and maintain ACQUITY UPLC[®] Fluorescence (FLR) detectors.

Intended use of the ACQUITY UPLC FLR detector

Waters designed the ACQUITY UPLC FLR detector to analyze many compounds, including diagnostic indicators and therapeutically monitored compounds.

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

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



Waters Corporation (Micromass UK Ltd.)
Floats Road
Wythenshawe
Manchester M23 9LZ
United Kingdom

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

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1 Theory of Operation

This chapter explains the theory and technology supporting the operation of the Waters® ACQUITY UPLC® Fluorescence (FLR) Detector and describes the instrument's features.

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Fluorescence theory

Fluorescence occurs when certain molecules absorb light at specific wavelengths, promoting the molecules to a higher energy state. As they return to their normal energy states, the “excited” molecules release their absorbed energy as photons.

Many organic compounds absorb light, but few fluoresce. LC systems that incorporate fluorescence detection effectively identify polyaromatic hydrocarbons, aflatoxins, vitamins, amino acids, and so on. Chemical derivatization methods extend fluorescence detection to some nonfluorescing compounds such as carbamate pesticides.

Fluorescence detection requires both the excitation/emission wavelengths, leading to a higher degree of sensitivity. As a result, this technique is useful for analysis requiring low detection limits.

Certain conditions can interfere with a compound’s ability to fluoresce, diminishing analytical performance:

- pH changes – The loss or gain of protons and their accompanying increase or decrease of charge affects an analyte’s electronic structure and can enhance or degrade fluorescence.
- Temperature changes – Fluorescence decreases as the sample temperature increases.
- Changes in the amount of dissolved oxygen – For some molecules, fluorescence is quenched (decreased) by the presence of dissolved oxygen.

Fluorescence detectors can be adapted to measure chemiluminescence, where a molecule without exposure to any excitation energy emits a low intensity signal. This type of detection can be accommodated by disabling the light source or (as in the case of this detector) enabling a shutter to stop any excitation light from reaching the flow cell.

The process of fluorescence detection involves an excitation source and the following processes:

- Filtering the source light
- Exciting the sample with filtered light
- Collecting and filtering the emitted fluorescence

- Measuring the emitted fluorescence
- Amplifying the emitted signal

Fluorescence detection

Overview

The scanning fluorescence detector illuminates a sample with a narrow band of high-intensity light. The detector then measures the low levels of fluorescence emitted by the sample. The emitted light is filtered, amplified, and converted to electrical signals that can be recorded and analyzed.

Excitation sources

A lamp that provides an intense, stable spectrum of light in the ultraviolet (UV) and visible ranges is the typical energy source used for fluorescence detection. The resulting fluorescence intensity is directly related to the intensity of the excitation spectrum. Thus high-sensitivity detectors use the most intense excitation source available.

Types of light sources

Common broadband excitation light sources include the xenon and mercury-xenon arc lamps. Xenon lamps are the preferred source for general-purpose fluorescence detectors. The mercury-xenon arc lamp in the FLR detector has the additional advantage of higher intensity at certain emission bands.

Excitation wavelength selection

The excitation wavelength of choice requires some source-light filtering. Modern detectors typically used a monochromator for the same purpose.

A monochromator is an adjustable device that you use to select wavelengths over a wide range of the spectrum. A grating monochromator uses a diffraction grating that passes only a small range, or bandwidth, of wavelengths. By moving the grating, you can select wavelengths within a particular range of wavelengths. A grating monochromator also passes fractions, or orders, of a selected wavelength. For example, if the monochromator is set to pass light energy at 600 nm, it also passes energy at

the second-order wavelength of 300 nm. A long-pass filter can absorb the higher-order energy produced by a monochromator. As the monochromator selects the excitation, it can also select the emission (radiated energy) can also be selected. Detectors with excitation and emission monochromators can scan holding one monochromator at a constant setting while varying the setting on the other. This type of operation is necessary when you are evaluating mixtures or analyzing chemical structures.

Exciting the sample

The broad band of high-intensity light from the lamp passes through a filter or monochromator, which selects a narrow band of wavelengths. This narrow band of light is then directed onto the flow cell where it excites the analytes as they pass through. Excitation wavelengths often correspond with the absorbance wavelength of the analyte.

Flow cell

The quartz flow cell minimizes the amount of stray light that can affect the measurement, and it maximizes the fluorescence signal. The sample compartment is arranged so that the fluorescence energy is collected at an angle perpendicular to the excitation (lamp) beam. This arrangement minimizes the effect of Rayleigh scatter on background light levels.

Cuvette cell

The FLR detector with the mercury-xenon lamp can deliver higher sensitivity than was possible using traditional HPLC fluorescence systems. Nevertheless, the new energy profile can require that the excitation spectrum of analytes be shifted significantly to match the energy bands of the mercury-xenon lamp, requiring you to optimize a traditional HPLC detection method for use with the ACQUITY detector. To determine optimized values, you can scan the excitation and emission wavelength ranges using the cuvette cell and view the spectrum plot in the ACQUITY Console or in Empower software using the Spectrum λ - λ plot function.

Measuring fluorescence

To measure fluorescence in the flow cell, the detector must balance the need for high selectivity (to distinguish very specific fluorescence wavelengths) with the need for high sensitivity (to measure low-fluorescence intensities).

Quantitation

Fluorescence is linear at low concentrations but can exhibit some nonlinearity at high concentrations.

Emission wavelength selection

A monochromator selects an emission wavelength.

Photomultiplier tube

The photomultiplier tube (PMT) produces a current proportional to the flux of photons emitted by the molecules in the flow cell.

Scanning

Detectors equipped with excitation and emission monochromators can easily scan a range of excitation or emission wavelengths. Changing the wavelength involves changing the monochromator setting. During a scan, the setting on one monochromator is held constant while the other monochromator scans a range of wavelengths.

Multichannel operation

Detectors equipped with excitation and emission monochromators can change the wavelength of the excitation and emission settings. In multichannel operation, both monochromators move rapidly between the selected wavelength pairs to produce multiple chromatogram traces. Multiple outputs can then derive additional information from a single separation.

Fluorescence data

Detectors report their data in units of fluorescence intensity (emission) or energy. The FLR detector also reports intensity using normalized units to compensate for variability between individual detectors and offset any age-related decrease in lamp intensity. When using normalized units, changes in gain improve the signal-to-noise ratio but do not change the peak response, conferring a high degree of bench-to-bench reproducibility of fluorescent signal measurements.

Emission units and normalization

The FLR detector offers two types of output units: emission and energy. Emission units are normalized to a standard water reference, and their magnitude is as independent of the PMT gain as possible. You can compensate for changes that normally influence the signal strength of fluorescence measurements, such as lamp or optics degradations, by periodically renormalizing to the standard water reference. Renormalizing reduces variations in fluorescence signal strengths from one detector to another.

The following equation calculates the emission units value (EU) at any time (t):

$$EU_t = (\text{PMTCounts}_t / \text{Gain}_t) \times (\text{Gain}_{\text{Raman}} / \text{Counts}_{\text{Raman}}) \times 100$$

where

$\text{Gain}_{\text{Raman}}$ and $\text{Counts}_{\text{Raman}}$ = values from the most recent execution of the normalize units function

PMTCounts_t and Gain_t = values at the time of data collection

Normalizing the emission units results in a water/Raman signal strength, at E_x 365 nm/ E_m 416 nm, of 100 emission units. The mercury-xenon spectrum output is not uniform over the detector's operating range, and low-UV wavelengths can degrade faster than normalization wavelengths.

Energy units

The alternative to emission units is energy units, which are similar to those used by traditional HPLC fluorescence detectors. They directly correlate to the anode current of the PMT, so they are directly influenced by the gain setting. All instrumental variables, such as lamp intensity, optics efficiency, and gain, directly influence the fluorescence emission signal strength. As a

result, energy units are less reliable. Nonetheless, when you must calculate energy units to conform to established protocols, use the following equation:

$$EU = \text{PMTCounts} \times K \times (\text{ReferenceCounts}_i / \text{ReferenceCounts}_r)$$

where K scales the maximum detectable fluorescence signal to 10,000 units.

References

Consult the following texts for additional information on fluorescence detection:

N. Ichinose, G. Schwedt, F. M. Schnepel, and K. Adachi, *Fluorometric Analysis in Biomedical Chemistry*, Chapter 5, Wiley-Interscience: New York, 1991.

E. S. Yeung, ed., *Detectors for Liquid Chromatography*, Chapter 5, Wiley: New York, 1986.

W. R. Seitz, in *Treatise on Analytical Chemistry*, 2nd ed., P. J. Elving, E. J. Meehan, I. M. Kolthoff, eds., Part I, Vol. 7, Chapter 4, Wiley: New York, 1981.

J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Plenum: New York, 1983.

S. G. Schulman, *Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practice*, Pergamon Press: New York, 1977.

J. D. Winefordner, S. G. Schulman, and T. C. O'Haver, *Luminescence Spectroscopy in Analytical Chemistry*, Wiley-Interscience: New York, 1972.

Detector description

The Waters ACQUITY UPLC FLR detector is a multichannel, tunable, fluorescence detector designed for UltraPerformance LC[®] system applications.

Features

The detector operates from 200 to 900 nm. It uses optics designed with an enhanced illumination system optimized for UPLC[®] performance. The following design features increase the optical throughput and sensitivity, resulting in an overall increase in the signal-to-noise ratio:

- High data rates – Ensure compatibility with narrow UPLC peaks.
- Small cell volume – Avoids bandspreading and maintains concentration.
- Single or multichannel mode – Monitors fluorescence at one or more discrete wavelength pairs.
- Integral erbium calibration reference – Ensures wavelength accuracy.
- Automatic second-order filter – Automatically engaged for wavelengths of 400 nm and greater and removed for wavelengths of 399 nm or less.
- Spectrum scan and storage display – Supports spectrum scan, display, and subtraction in addition to standard tunable fluorescence functionality.
- Normalized emission units – Enhance unit-to-unit reproducibility.
- Idle mode – Closes a shutter to prevent degradation of the optics.
- 3D mode – Dynamically scans the emission and/or excitation grating through a selectable wavelength range to monitor the fluorescence of a series of wavelengths, enabling the production of 3D data.
- Spectrum lambda-lambda mode – Creates a spectrum that characterizes analyte fluorescence across the excitation and emission axes.

Principles of operation

To use the detector effectively, become familiar with its optical and electronic design and the theory and principles of its operation.

- Optics
- Wavelength verification and test
- Flow cell
- Electronics

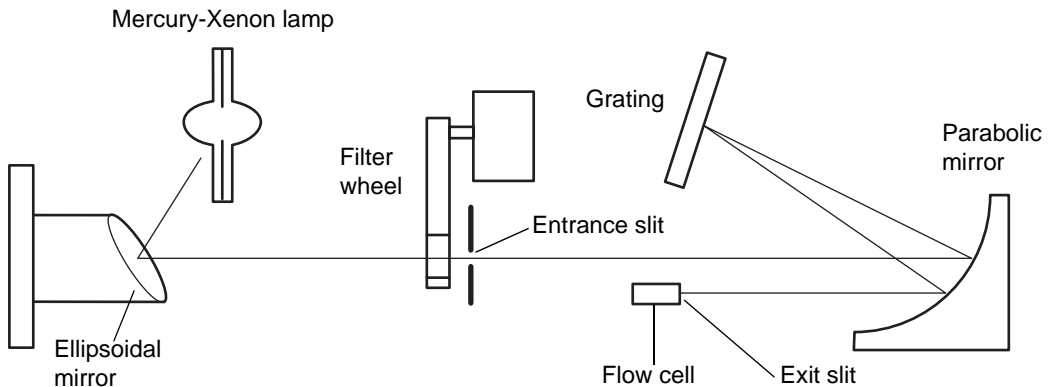
Detector optics

The optics are based on a pair of tunable monochromators and include the following parts:

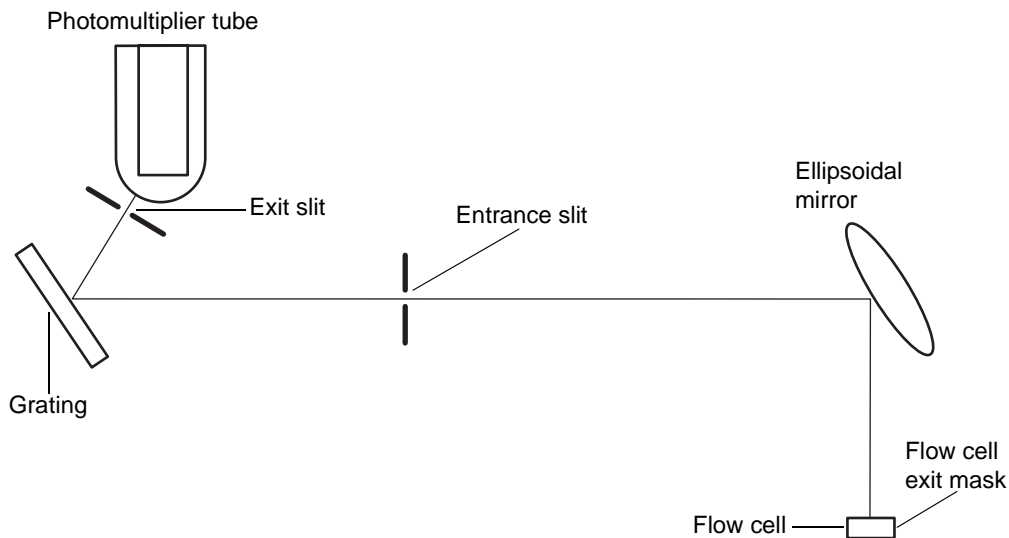
- Mercury-xenon arc lamp
- Two ellipsoidal mirrors and one parabolic mirror
- Shutter, wavelength calibration filter, and second-order filter
- Entrance slits
- Exit slits
- Blazed, plane, and concave holographic diffraction gratings
- Photomultiplier tube (PMT)
- Waters axially illuminated flow cell

The following diagrams show the optics assembly light paths and components.

Excitation monochromator optics assembly



Emission monochromator optics assembly



Optics assembly light path

The detector, optimized for UPLC, demonstrates superior performance by employing several unique design elements. Its novel flow cell design minimizes stray background light and increases the detectability of low-level signals. Keeping the optics simple tends to minimize loss of signal and maximize throughput.

Light source

The detector uses a high-intensity 150-watt mercury-xenon arc lamp as its source. The emitted light passes through the excitation monochromator to flood the aperture of the flow cell. The lamp light is collected by an elliptical mirror behind the lamp, its center of curvature oriented toward the lamp's bright spot.

Excitation monochromator

The detector uses a monochromator to select the appropriate excitation wavelengths defined by its geometry. The grating can rotate quickly, responding to multiple excitation wavelengths and/or scanning.

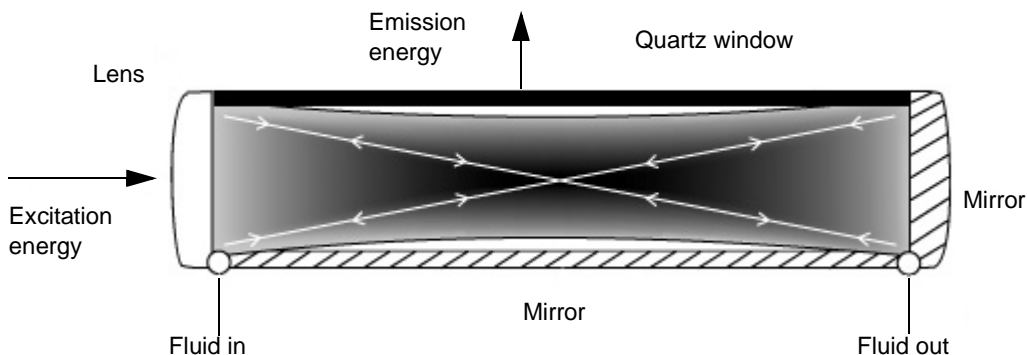
Emission monochromator

The light emitted by the sample travels from the top of the flow cell into the emission optics. The emission optics are positioned at right angles to the excitation source to minimize the possibility of stray light reaching the PMT. The emission monochromator selects the appropriate emission wavelengths.

Axially illuminated flow cell

The flow cell design incorporates an axially illuminated, fused-quartz flow cell.

Axially illuminated flow cell



The excitation energy is focused on a geometrically matched mirror whose shape is opposite that of the excitation energy entrance lens. Excitation energy is reflected along the flow cell axis and back, effectively doubling the pathlength of the cell. The increased pathlength in turn provides superior sensitivity compared to traditional fluorescence detectors.

PMT calibration

You control the sensitivity of the detector by the gain setting, which increases the voltage to the PMT to amplify and increase response. The gain is achieved by controlling the high-voltage supply to the PMT. After the assembly and alignment of the detector and whenever the PMT or any PC boards are replaced, Waters personnel calibrate PMTs using an onboard service diagnostic function.

PMT sensitivity

After calibrating the PMT, you must choose a gain setting for it prior to a chromatographic injection. Saturation, which occurs when the sample concentration is high or the experimental mobile phase has a high background, is always a concern—even when the PMT gain setting is at the lowest level. For this reason, the ACQUITY UPLC FLR detector has an Auto Optimize Gain diagnostic function that lets you adjust the granularity of the gain.

Filtering noise

The detector uses a digital filter to minimize noise.

Lower time constant settings produce these effects:

- Narrow peaks appear with minimal peak distortion and time delay.
- Very small peaks become harder to discriminate from baseline noise.
- Less baseline noise is removed.

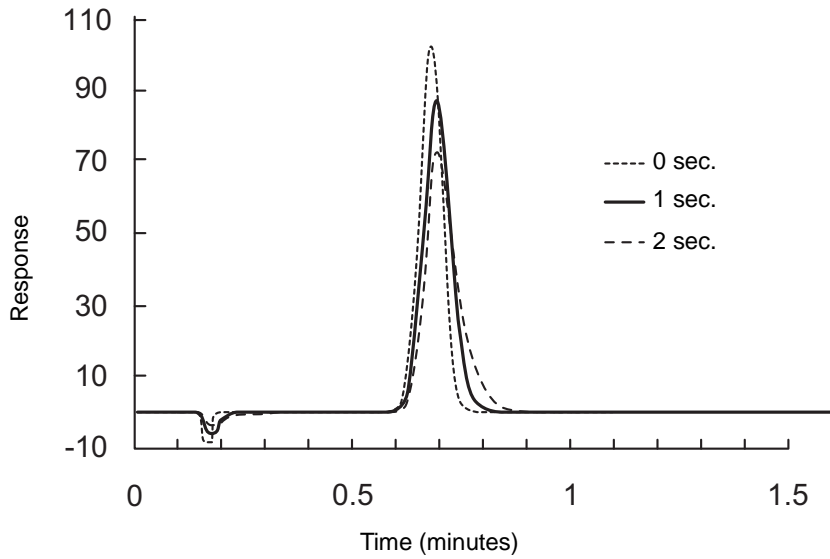
Higher time constant settings produce these effects:

- Greatly decreased baseline noise
- Shortened and broadened peaks

The software includes fast, normal, or slow filtering constants at each data rate that are appropriate for high-speed or high-sensitivity applications, respectively.

The following figure shows the relationship between increased time-constant and response times.

Effect of filter time constant



Electronics

The electronics consist of the following components:

- **Preamplifier board** – Collects and processes the analog input signals from the PMT and photodiode to the microprocessor for further signal conditioning. Sample and reference signals are integrated and A/D conversion is performed simultaneously. This component ensures the best rejection of common mode noise in the two beams, leading to a quiet baseline.
- **Personality board** – Receives inputs from the preamplifier board and external events. It also provides control of the optics positioning subsystems and the lamp power supply.
- **CPU board** – Contains the digital signal processor, communication ports, nonvolatile (battery backed-up) RAM, and flashable RAM space in which the firmware resides.
- **Ethernet communications interface** – Enables the detector to communicate with data system software.

- Lamp power supply – Provides stable mercury-xenon lamp operation.
- DC power supply – Provides voltage for the analog and digital circuitry of the detector.

Wavelength verification and test

The mercury-xenon arc lamp and the integral erbium filter exhibit peaks in the transmission spectrum at known wavelengths. Upon startup, the detector waits 5 minutes for the mercury-xenon lamp to warm and stabilize. The detector verifies calibration by comparing the locations of these peaks with calibration data stored in memory. If the results of this verification differ from the stored calibration by more than ± 2.0 nm, the detector displays a wavelength verification failure message. This message indicates a manual wavelength calibration is necessary. The detector verifies, rather than recalibrates, to avoid errors that can occur if the flow cell contains residual materials. Calibration requires a clean flow cell and transparent mobile phase. You can initiate a manual wavelength calibration anytime to replace the previous calibration data with new data.

Note: The combined wavelength accuracy specification of the detector is ± 3.0 nm, but the wavelength accuracy of each grating is held to ± 2.0 nm.

When the detector runs continuously, you should perform wavelength verification weekly by turning it off and then on again, or perform the calibrate wavelength function through the console. The verification tests require 5 minutes of lamp warmup time to stabilize the lamp.

Operational modes

The detector operates in single or multichannel mode, allows spectrum scanning using a flow cell, and provides Difference and MaxPlot functions.

Single-channel mode

The detector defaults to single-channel mode, monitoring a single channel for an excitation/emission wavelength pair. You can specify the excitation wavelength between 200 and 890 nm on channel A.

In single-channel mode, the detector automatically engages the second-order filter for excitation wavelengths of 400 nm and longer and removes it for wavelengths shorter than 399 nm. The second-order filter is an optical filter

that blocks unwanted UV light from reaching the diffraction grating, which can interfere with fluorescence detection of 400 nm and longer.

Selecting the appropriate sampling rate

A sufficient number of points must fall across a peak to define its shape. For this reason, the definition between peaks is lost at very low sampling rates. Empower software uses the index of the data point closest to the end time, minus the index of the data point closest to the start time, to calculate the Points Across Peak value for each integrated peak in the chromatogram.

Tip: The Points Across Peak value appears in the Peaks table, at the bottom of the Review Main window. If the Points Across Peak field is not visible, right-click anywhere in the table, and then click Table Properties. Click the Columns tab, and then scroll down to find the Points Across Peak field. Clear the check box, and then click OK.

If the Points Across Peak value for the narrowest peak of interest is less than 25, you must specify a higher sampling rate in the instrument method. If the value is greater than 50, specify a lower sampling rate in the instrument method.

Set the sampling rate to the lowest value required to achieve 25 or more points across the narrowest peak. Excessively high sampling rates exhibit higher noise levels.

Multichannel mode

In multichannel, or multiwavelength, mode, the detector monitors two or more excitation/emission wavelength pairs. The sampling frequency range is reduced, limiting the use of this mode to a more standard chromatography, where peaks are not excessively narrow. You can use multiwavelength mode to obtain additional information about an analyte by running a Difference Plot or a MaxPlot. You can select up to four excitation wavelengths from 200 to 890 nm and up to four emission wavelengths from 210 to 900 nm. For the best signal-to-noise ratio, set a gain that maximizes the dynamic range of the electronics. A gain that is too high overloads the pre-amplifier, resulting in flat-topped peaks and a warning alarm.

MaxPlot

The detector allows you to obtain a MaxPlot in multichannel mode. The MaxPlot function monitors fluorescence at the selected excitation/emission wavelength pairs and plots the maximum fluorescence signal value for each sample component. The MaxPlot outputs the greater of the fluorescence values on the selected channels.

See also: ACQUITY UPLC Console online Help.

Difference plot

The detector allows you to obtain a difference plot in multichannel mode. The difference plot function monitors fluorescence at user-selected excitation/emission wavelength pairs and plots the difference in signal value between them.

See also: ACQUITY UPLC Console online Help.

3D mode

The detector collects 3D data (either emission or excitation scans) and stores them in files from which you can extract 2D chromatograms, select and optimize wavelength selections for specific applications, and create searchable libraries. The resolution is typically higher than the stopped-flow scan modes.

Spectrum lambda-lambda mode

Using the FLR detector's spectrum lambda-lambda (λ - λ) mode, you can determine fluorescence maxima for unknowns and use them to quickly develop traditional retention-time-based chromatographic methods. The spectrum λ - λ mode creates a spectrum that characterizes analyte fluorescence across the excitation/emission axes. Under static flow conditions, the detector scans a specified range of emission wavelengths over a specified range of excitation wavelengths. Fluorescence is thus plotted against excitation/emission wavelength axes, and the results appear in a data file.

Excitation wavelengths appear along the axis that usually displays time, and the wavelength values are reduced by a factor of 10 to fit onto the time axis. The axis continues to be labeled as “minutes”.

Requirement: An optional cuvette cell is required when scanning in lambda-lambda mode.

Spectrum scanning

You can use the detector as a fluorometer to acquire spectra and store them as a file. The major difference between this detector and a double-beam spectrophotometer is that this detector employs only one flow cell rather than a simultaneous sample and reference flow cell pair. The detector obtains a fluorescence spectrum by performing the following types of scan on the flow cell:

- Zero scan – Characterizes the baseline absorbance spectrum of a solvent.
- Excitation sample scan – Subtracts the zero scan, so the displayed or charted results are of the sample's excitation spectra only.
- Emission sample scan – Subtracts the zero scan, so the displayed or charted results are of the sample's emission scan only.

To obtain an excitation or emission spectrum of a sample, run a zero scan followed by the appropriate sample scan. Typically, a zero scan is run with pure solvent. A sample scan is usually run with the analyte dissolved in the same solvent.

Lamp energy and performance

In conventional designs of fluorescence detectors, the signal-to-noise performance of the instrument is directly proportional to the lamp energy input to the instrument. Lamp energy input to the detector can be affected by

- age and efficiency of the lamp.
- improperly maintained optics and/or flow cell.
- normal degradation of optical components (including the PMT).

Optical components degrade slowly over time. In conventional fluorescence detectors, response increases by incrementing the PMT gain. However, the response of the sample varies with energy throughput. If excitation energy is degraded, peak response is degraded. If excitation intensity diminishes, peak response decreases and noise increases.

During normal operation, lamps are commonly replaced when the reference energy falls below a user-set threshold. The useful lamp life depends on your requirements for noise performance.

Tip: Inspect the detector's general condition when you replace lamps.

Predicting when the detector's performance degrades to an unacceptable level based solely on reference energy is unsatisfactory. Each user's analyses will require different levels of sensitivity. Verifying reference energy alone to evaluate performance assumes that every lamp has the same longevity, degradation patterns, and spectral output characteristics. To reduce this uncertainty, Waters designed the detector to operate as independently of lamp output as possible. After the unit has verified the calibration of the monochromator, the instrument evaluates the energy levels in a number of characteristic regions across the spectrum. The instrument adjusts the integration time of the front-end electronics to maximize the signal within these regions. The intent is to maintain a high signal-to-noise ratio and operate with a clean signal. In this way, the instrument's sensitivity to lamp energy is virtually eliminated as a major contributor to performance.

Ultimately, the detector's performance is a function of each unique application requirements. Signal-to-noise measurements are the best way to evaluate performance and set the boundaries for acceptable operational sensitivity limits.

The FLR detector source lamp is warranted to light and pass startup diagnostic tests for 1000 hours or 1 year from the date of purchase, whichever comes first. The ACQUITY UPLC console allows you to record lamp usage and report the lamp serial number.

Auto-optimize gain

Proper PMT gain setting maximizes the signal on the internal analog-to-digital converter without exceeding its potential limit. If you specify too high a gain, the fluorescence emissions overload the signal collection electronics. Too low a gain reduces sensitivity to emission signals, degrading signal-to-noise ratios. The detector therefore requires you to specify a gain setting for the PMT before you inject a sample. However, before the injection, you cannot know the magnitude of your fluorescence signal. Users traditionally resolve this difficulty by running several injections to determine a suitable gain setting, a tedious process, especially when they run timed event changes in gain and/or wavelength.

The Auto-Optimize Gain diagnostic function lets you run a trial chromatogram and displays the ideal gain values. The reported values are based on an algorithm that ensures a 2× margin against overloading the PMT and its associated electronics with variations in fluorescence intensity for concentrated samples. In the case of timed event changes in gain and/or

wavelength, the report reflects adjusted values representing the ideal gain setting for each critical timed event region. Incorporate the reported gain values in the method, including its timed event table, to optimize the method's performance.

The detector also monitors the maximum fluorescence signal level throughout the run. When you use the analog outputs during data collection, it displays a minimum EUFS value that applies to the entire chromatogram. Like the ideal gain value, the EUFS value assumes a 2× margin to account for any variations in fluorescence intensity. Based on this report, adjust the gain values in the method, including its timed event table, to optimize the performance of the method.

The detector also monitors the maximum fluorescence signal level throughout the entire run. It recommends a minimum EUFS value, which applies to the entire chromatogram and appears when you use the analog outputs during data collection. This value is also computed assuming a 2× margin for error.

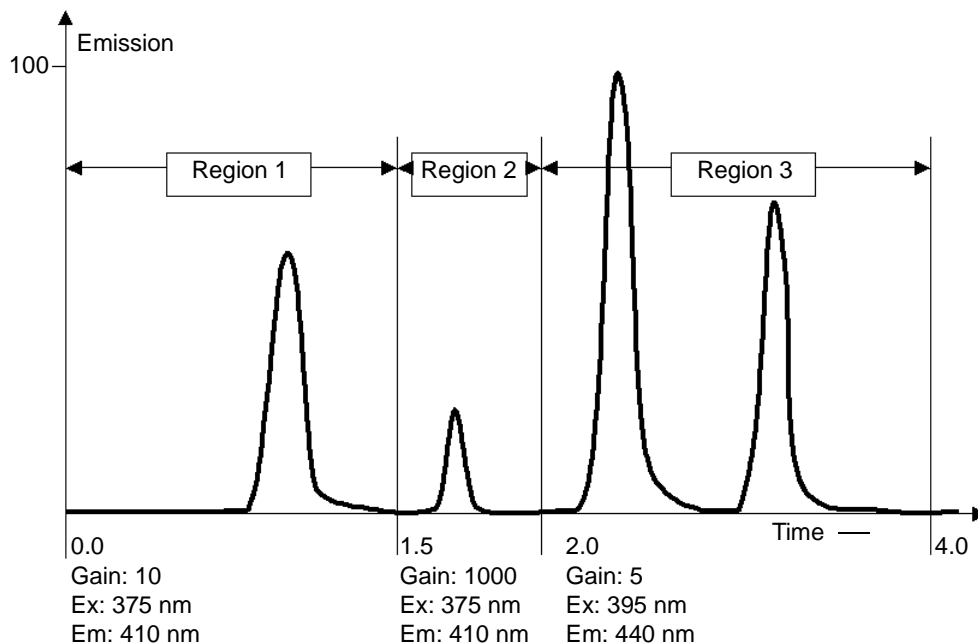
Method optimization

You can download a method that includes timed event changes. The timed event changes that alter gain, excitation wavelength, or emission wavelength are critical “light condition” changes. These are the points at which the signal peak maximum search gets renewed. You must therefore enter any timed event gain changes at strategic points before peaks to improve the detector's sensitivity to them. The goal is to provide a retention time demarcation point at which a gain change could be tolerated without disrupting the integration of peaks in the chromatogram. Before you run the Auto-Optimize Gain diagnostic function, you must set the initial conditions. No timed events are necessary. This means, however, that the detector will recommend only one gain value setting for all peaks in the chromatogram and no segregated peak region optimization.

Example of recommended method development approach

Use a method with two timed event changes to optimize the chromatogram shown below.

Gain-optimized chromatogram



The first gain setting change could occur at 1.5 minutes, just before the small peak that is best detected at a gain of 1000. The next change could occur at 2.0 minutes, for the required wavelength pair change. Initial gain setting or conditions are not critical. The only requirement for the first timed event is that some gain setting takes place. An example of an initial method table is shown below.

Example of method development

Time (min)	Event
Initial (0.0)	Excitation = 375 nm, Emission = 410 nm, Gain = 100
1.5	Gain = 1
2.0	Excitation = 375 nm, Emission = 410 nm (no need to change gain here)

After you run the Auto-Optimize Gain diagnostic function, the detector displays recommended gain values.

Recommended gain values

EUFS: 2000 event time (min.)	Best gain
0.0 (Initial)	10
1.5	1000
2.0	5

Tips:

- The previous table contains the best gain values optimized with a 2× margin for error that holds half its capacity in reserve for unanticipated fluorescence signal fluctuations.
- The magnitude of emission units is independent of the gain, so changing gain does not affect emission unit values. However, when you use sample energy units, changing the gain does affect the magnitude of the output signal.

Ensuring gain optimization for each peak of interest

Refer to the [figure “Gain-optimized chromatogram” on page 1-20](#). If you use only one timed event (wavelength pair change at 2 minutes for peaks 3 and 4), the recommended gain table is as follows.

Recommended gain values with a single timed event change

EUFS: 2000 event time – min.	Best gain
0.0 (Initial)	10
2.0	5

The gain for Region 2 is determined by the maximum signal level in Region 1. Therefore, a gain of only 10 would be used from time 0.0 to time 2.0, but the small peak may not be adequately resolved at this setting. If the detector did find it, the peak area integration would be far less accurate due to higher baseline noise. Failure to program a gain change at a strategic point in the chromatogram results in an inferior approach to method development.

Startup diagnostic tests

The detector runs a series of startup diagnostic tests and posts an error message if any test fails. The startup diagnostic tests are as follows:

- Central processing unit (CPU) test
- Serial communication interface (SCI) test
- Electrically erasable programmable read-only memory (EEPROM) test
- RAM test
- Application program checksum verification
- Lamp test
- Photodiode test
- PMT test
- Optics test/Wavelength verification

Mobile-phase solvent degassing

Mobile-phase difficulties account for at least 70% of all liquid chromatographic problems. Using degassed solvents is important, especially at excitation wavelengths shorter than 220 nm. Bubbles in the flow cell adversely affect detector performance. The ACQUITY UPLC system provides a degasser to remove most of the gas (air) from solvents. Degassing provides

- reproducible fluorescent response.
- stable baselines and enhanced sensitivity.
- reproducible retention times for eluting peaks.
- reproducible injection volumes for quantitation.
- stable pump operation.

Wavelength selection

In fluorescence, if the excitation monochromator is set below the UV cutoff of a mobile-phase component, the solvent absorbs some of the available excitation light intensity, which in turn reduces the fluorescence emission response for the sample. For a complete list of UV cutoff ranges for common solvents and common mixed mobile phases, refer to Appendix C of the *ACQUITY UPLC System Operator's Guide*.



Warning: Using incompatible solvents can cause severe damage to the instrument and injury to the operator.

2 Setting Up the Detector

Contents:

Topic	Page
Before you begin	2-2
Installing the detector	2-3
Plumbing the detector	2-5
Making Ethernet connections	2-12
Connecting to the electricity source	2-16

Before you begin

Requirement: To install the detector, you must know how to set up and operate laboratory instruments and computer-controlled devices and how to handle solvents.

Tip: Use this guide in conjunction with the ACQUITY UPLC system documentation and online Help.

Before installing the detector, ensure that

- it is not situated under a heating or cooling vent.
- the required components are present.
- none of the shipping containers or unpacked items are damaged.

Environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	20 to <95%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	0 to <95%, noncondensing

If you discover any damage or discrepancy when you inspect the contents of the cartons, immediately contact the shipping agent and your local Waters representative.

Customers in the USA and Canada can report damage and discrepancies to Waters Technical Service (800 252-4752). Others can phone their local Waters subsidiary or Waters corporate headquarters in Milford, Massachusetts (USA), or visit www.waters.com and click Waters Division.

For complete information on reporting shipping damages and submitting claims, see *Waters Licenses, Warranties, and Support Services*.

Installing the detector

To install the detector

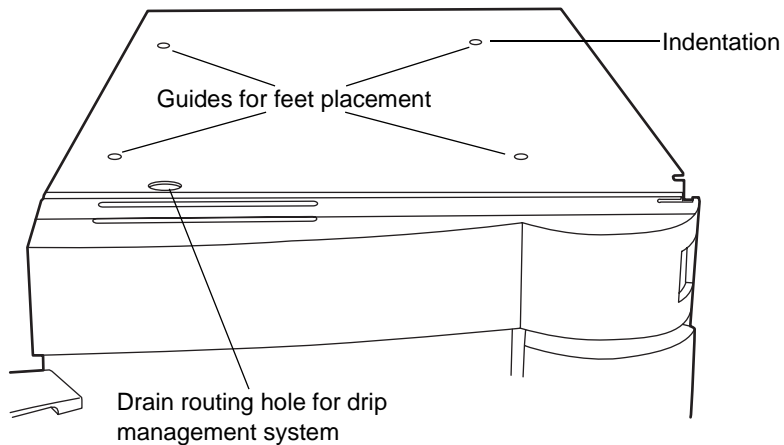


Warning: To avoid injury, Waters recommends that two people lift the ACQUITY UPLC FLR detector.

1. Place the detector atop the column heater, ensuring that the feet are properly positioned in the indentations of the column heater.

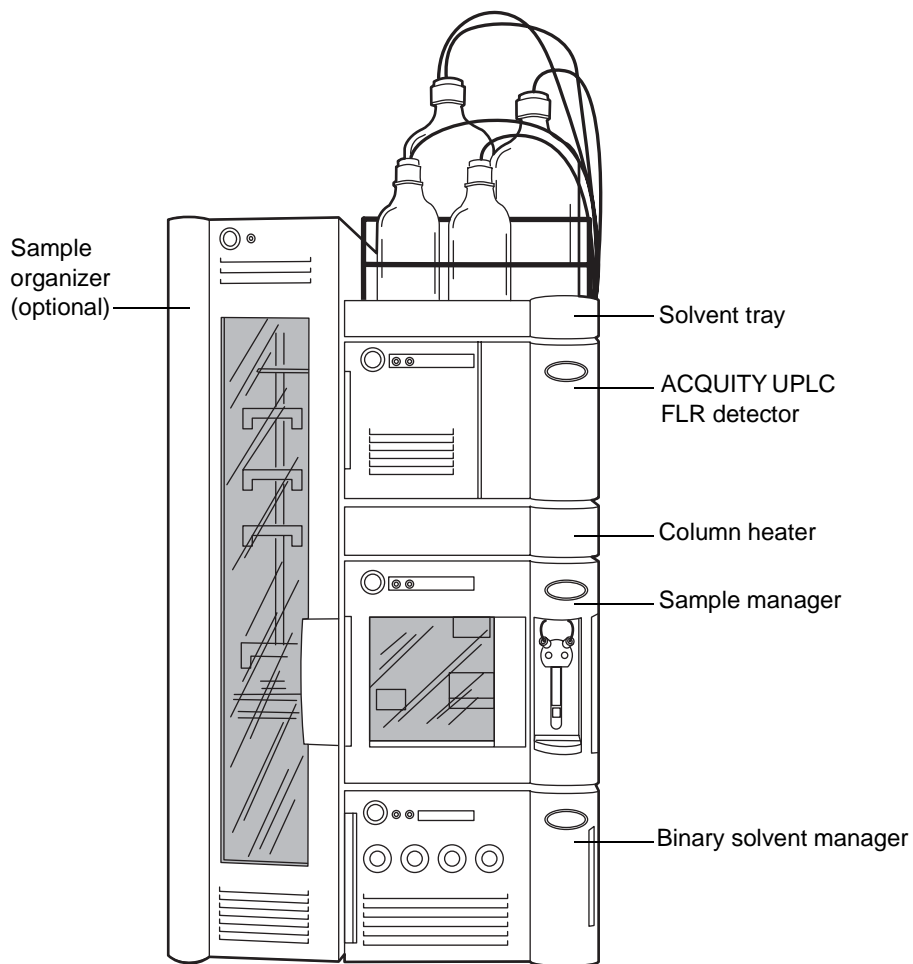
Result: The detector's drip tray is now properly aligned over the drain routing hole on the top, left-hand side of the column heater.

Proper placement for drip management system



2. Place the solvent tray module atop the detector.

ACQUITY UPLC FLR detector installed in ACQUITY UPLC system



Plumbing the detector



Warning: Using incompatible solvents can cause severe damage to the instrument and injury to the operator. Refer to Appendix C of the *ACQUITY UPLC System Operator's Guide* for more information.



Caution: To prevent contamination, wear powder-free, nonlatex gloves when plumbing the detector.

Plumbing the detector involves connecting the flow cell and installing a backpressure regulator, if necessary.

Although the inline degasser removes most of the gas (air) from solvents, some gas is reintroduced during partial loop injections. Under pressure, this gas remains in solution. However, because the postcolumn pressure is normally much lower than the precolumn pressure, the gas can come out of solution and produce an unstable baseline characterized by large, unexpected spikes.



Caution: When installing the large-volume flow cell on the FLR detector, do not use the standard pressure regulator shipped with the detector. That regulator's 1724 kPa (17 bar, 250 psi) set point, which exceeds the large-volume flow cell's 1000 kPa (10 bar, 145 psi) pressure limit, can damage the flow cell. Use the backpressure regulator shipped with the large-volume flow cell, which has a set point of 689 kPa (7 bar, 100 psi).

A backpressure regulator maintains a minimum postcolumn pressure of 1724 kPa (17 bar, 250 psi), eliminating postcolumn outgassing and ensuring a smooth baseline.

Requirements:




Caution: To prevent flow cell breakage, do not exceed the standard flow cell's maximum allowable pressure of 3447 kPa (34 bar, 500 psi). If you have installed the large-volume flow cell, do not exceed that flow cell's 1000 kPa (10 bar, 145 psi) pressure limit.

- Because the standard flow cell is pressure-rated to a maximum of 3447 kPa (34 bar, 500 psi), the FLR detector must be the last detector in the system. The backpressure regulator is required for optimum performance.
- If a mass spectrometer is to be used in the ACQUITY UPLC system, a flow splitter must be installed ahead of the FLR detector.

Recommendation: To avoid particulate contamination in the flow cell, flush columns before connecting them to the detector.

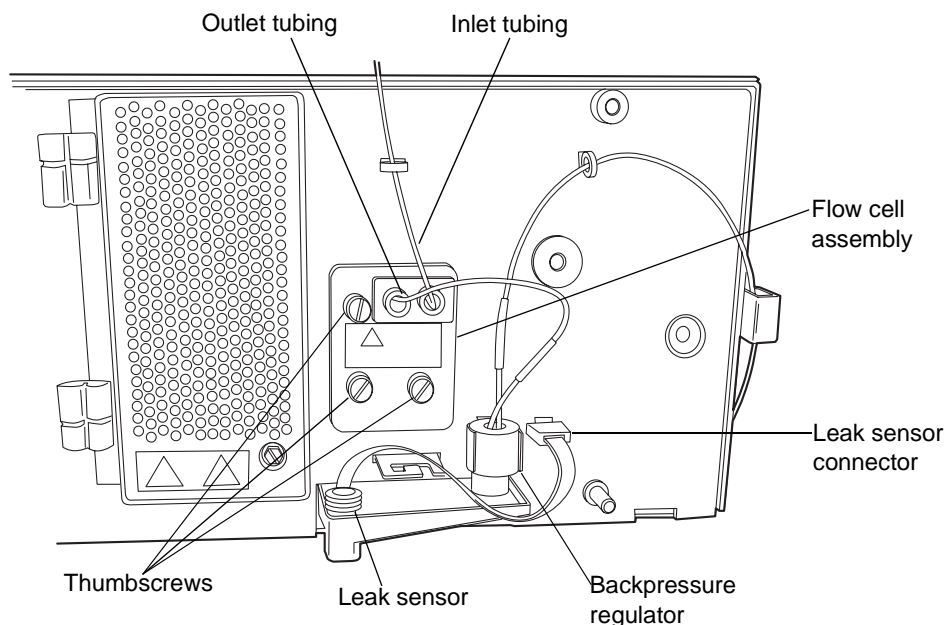
See also: "Installation recommendations for fittings" in the *ACQUITY UPLC System Operator's Guide*.

To plumb the detector

Recommendation: If the detector is already powered on, in the console, select FLR Detector from the system tree and click  (Lamp Off) to extinguish the lamp.

1. Open the detector's front panel door.

ACQUITY UPLC FLR detector with front panel door open

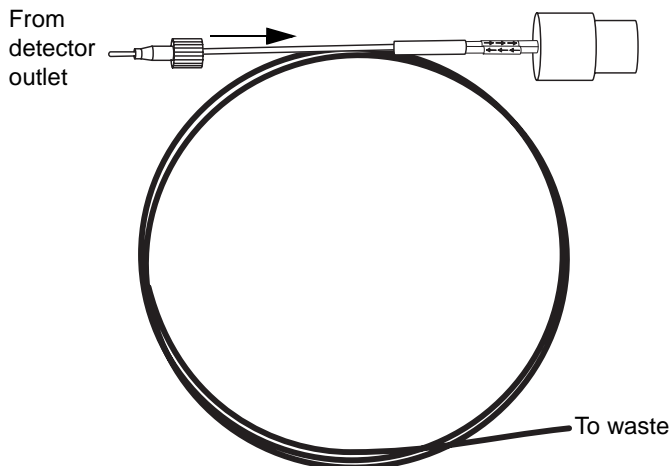


Caution: When installing the large-volume fluorescence flow cell on the ACQUITY UPLC FLR detector, do not use the standard pressure regulator shipped with the detector. That regulator's 1724 kPa (17 bar, 250 psi) set point, which exceeds the large-volume flow cell's 1000 kPa (10 bar, 145 psi) pressure limit, can damage the flow cell. Use the backpressure regulator shipped with the large-volume flow cell, which has a set point of 689 kPa (7 bar, 100 psi).

2. Remove the protective cover from the PEEK cell inlet tubing, and connect the tubing to the flow cell inlet. Confirm that the label on the tubing matches the type of detector and flow cell in your system.

3. Attach the short length of outlet tubing from the backpressure regulator to the outlet of the flow cell.

Backpressure regulator

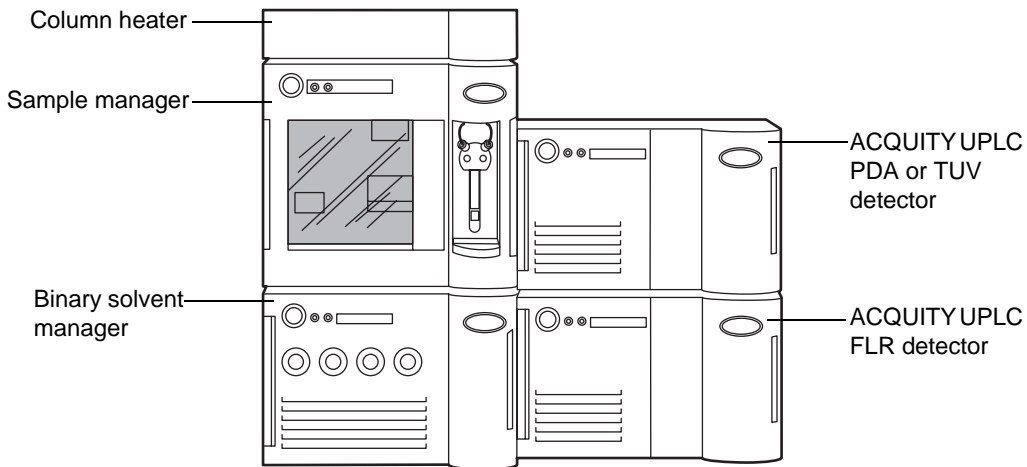


4. Route the long end of the outlet tubing from the backpressure regulator, through the channel clips along the front, right-hand side of the system, and into a suitable waste container.

Installing the multidetector drip tray

If your ACQUITY UPLC system has more than one detector, you must install the multidetector drip tray.

ACQUITY UPLC FLR detector installed in a split ACQUITY UPLC system



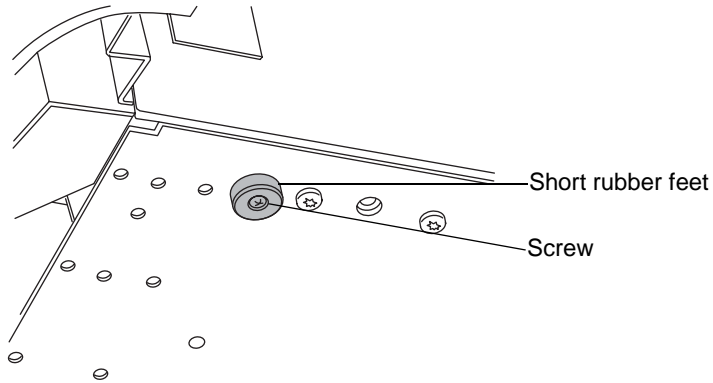
Required materials

- Multidetector drip tray kit
- T20 TORX[®] driver

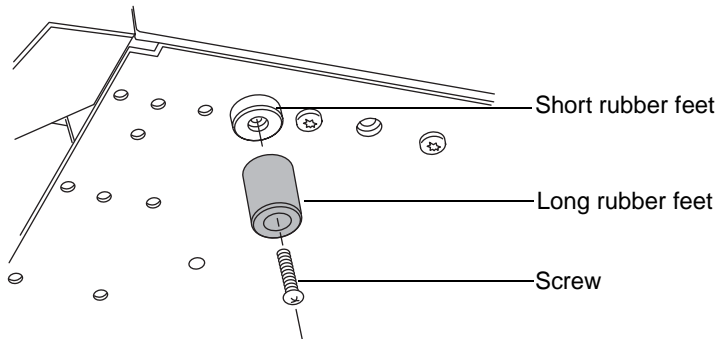
To install the drip tray

1. Turn the ACQUITY UPLC FLR detector so that it is resting on its left-hand side.

2. Using the T20 TORX driver, remove the screws that secure the four feet to the bottom of the detector.

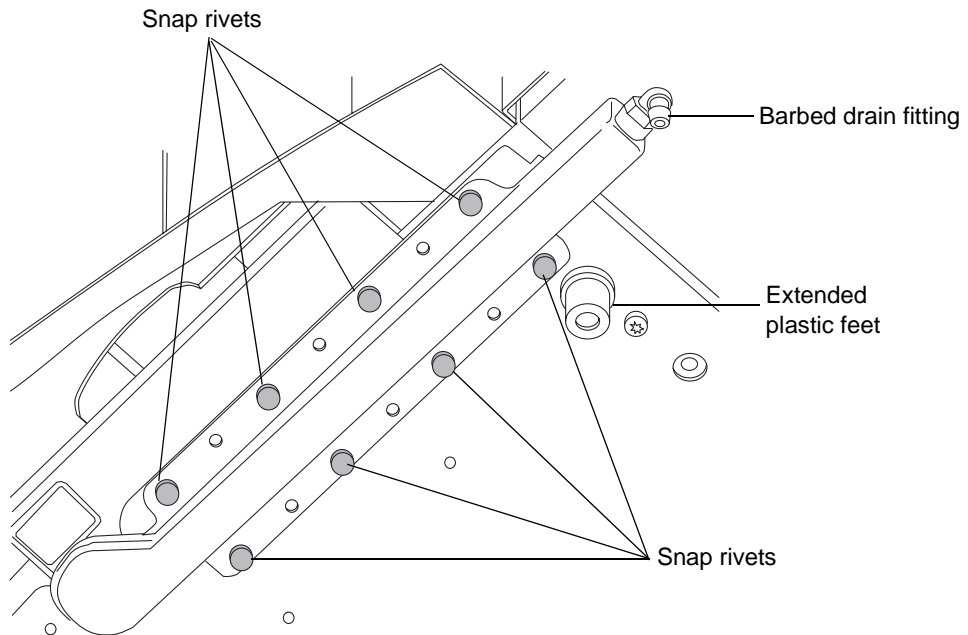


3. Using the T20 TORX driver and 30-mm screws, fasten the short and then the long rubber feet onto the bottom of the detector.



4. Secure the drip tray to the bottom of the detector by inserting the snap rivets in the unobstructed holes.

Tip: The number of rivets required depends on the type of detector.



5. Turn the FLR detector to its original position.
6. Return the PDA or TUV detector to its original position atop the FLR detector.
7. Slide a waste line over the barbed drain fitting located on the right-hand side of the drip tray, and route it to a suitable waste container.

Making Ethernet connections

To make Ethernet connections

1. Unpack and install the preconfigured data system workstation.
2. Connect one end of one Ethernet cable to the network switch, and then connect the other end to the Ethernet card on the workstation.

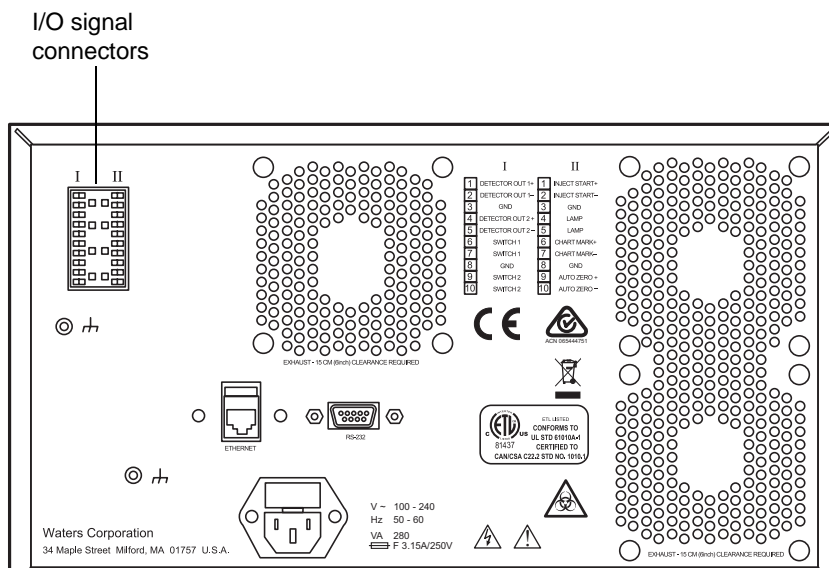
Tip: On preconfigured systems, the Ethernet card is identified as the Instrument LAN card.

3. Connect one end of one Ethernet cable to the back of the detector, and then connect the other end to the network switch.

I/O signal connectors

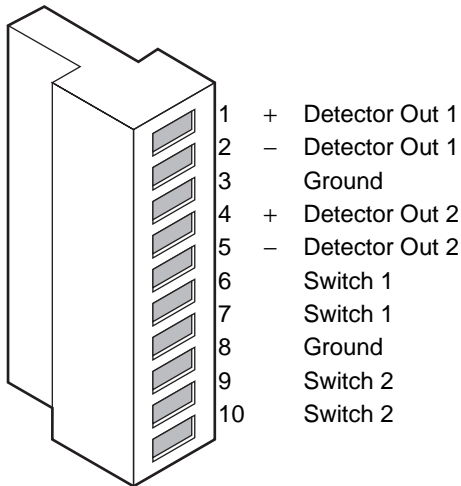
The detector's rear panel includes two removable connectors that hold the screw terminals for I/O signals. These connectors are keyed so that they can receive a signal cable inserted only one way.

Location of I/O signal connectors

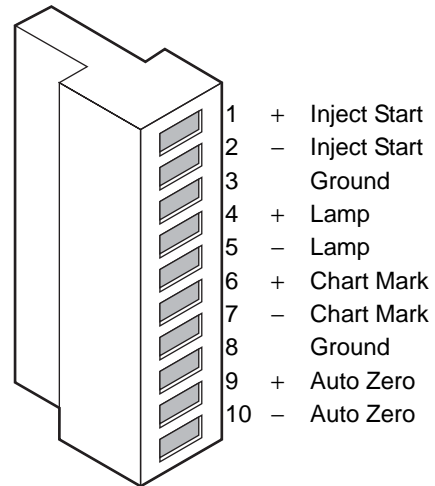


I/O signal connectors

Connector I (inputs and outputs)



Connector II (inputs)



FLR detector analog-out/event-in connections

Signal connections	Description
Inject Start	Activates timed events by triggering the run-time clock to start. This connection should not be used.
Lamp	Allows an external device to turn off the mercury-xenon lamp.
Chart Mark	Adds a chart mark (at 10% of full scale) to one or both analog output channels (Detector Out 1 and Detector Out 2).
Auto Zero	Performs Auto Zero function on both channels (Detector Out 1 and Detector Out 2).
Detector Out 1	1-V full-scale analog output signal of Channel A (scaled to the current EUFS setting).
Detector Out 2	1-V full-scale analog output signal of Channel B (scaled to the current EUFS setting).
Switch 1 (2)	Can be controlled by threshold and timed events.
Switch 2 (2)	Can be controlled by threshold and timed events.

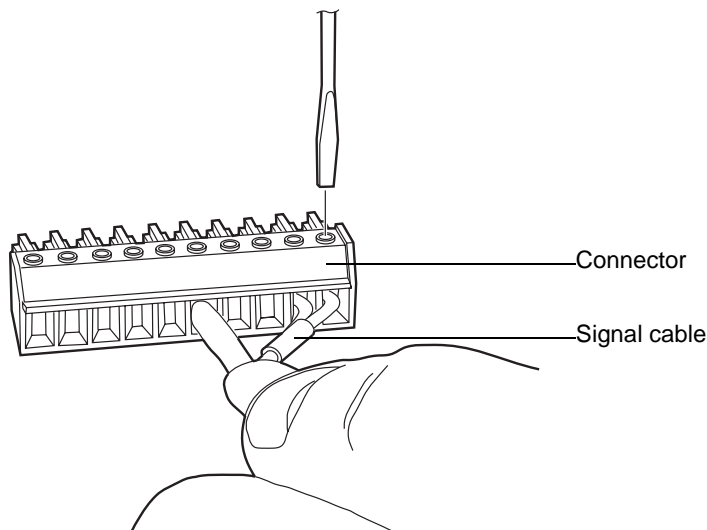
Signal connections

Refer to the signal connection location shown on the silk-screened label on the rear panel of each instrument.

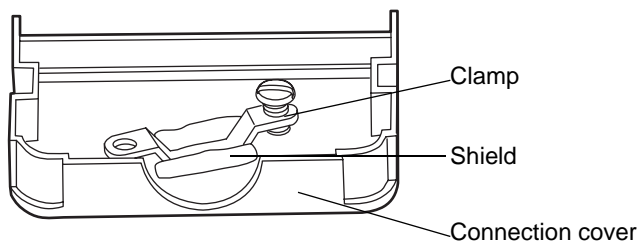
Requirement: To meet the regulatory requirements of immunity from external electrical disturbances, you must install connection covers over the signal connectors.

To make signal connections

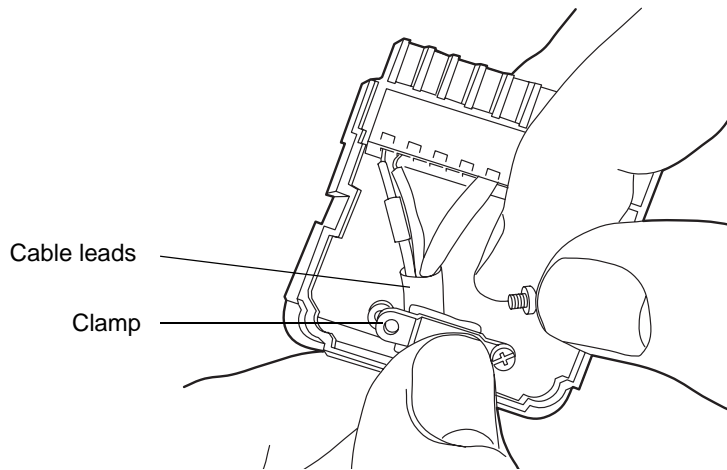
1. Attach the positive and negative leads of the signal cable to the connector.



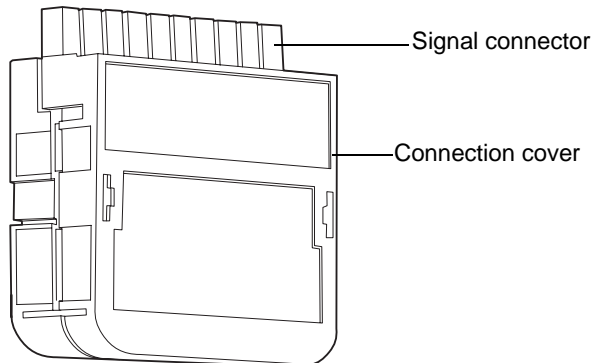
2. Slide the clamp (with the bend facing down) into the protective shield.
3. Insert the clamp and shield (with the bend facing down) into the connection cover and loosely tighten with 1 self-tapping screw.



4. Insert the connector with the signal cable into the connection cover, position the clamp over the cable leads, and then tighten the clamp into place with the second self-tapping screw.



5. Place the second connection cover over the first cover and snap it into place.



Connecting to the electricity source

The FLR detector requires a separate, grounded electricity source. The ground connection in the electrical outlet must be common and connected near the system.



Warning: To avoid electrical shock:

- Use power cord SVT-type in the United States and HAR-type (or better) in Europe. For other countries, contact your local Waters distributor.
- Power-off and unplug the detector before performing any maintenance on the instrument.
- Connect all components of the ACQUITY UPLC system to a common ground.

To connect to the electricity source

Recommendation: Use a line conditioner or an uninterruptible power supply (UPS) for optimum long-term input voltage stability.

1. Connect the female end of the power cord to the receptacle on the rear panel of the detector.
2. Connect the male end of the power cord to a suitable wall outlet.

Alternative: If your system includes the optional FlexCart, connect the female end of the FlexCart's electrical cable (included in the startup kit) to the receptacle on the rear panel of the detector. Connect the hooded, male end of the FlexCart's electrical cable to the power strip on the back of the cart. Finally, connect the power strip's cable to a wall outlet operating on its own circuit.

3 Preparing the Detector for Operation

Contents:

Topic	Page
Starting the detector	3-2
Performing a run	3-6
Installing the cuvette cell	3-8
Shutting down the detector	3-10

Starting the detector



Warning:

- Always observe Good Laboratory Practices when you use this equipment and when you work with solvents and test solutions. Know the chemical and physical properties of the solvents and test solutions you use. See the Material Safety Data Sheet for each solvent and test solution in use.
- Using incompatible solvents can cause severe damage to the instrument and injury to the operator. Refer to Appendix C of the *ACQUITY UPLC System Operator's Guide* for more information.



Warning: Explosion hazard. The flash point is the lowest temperature at which a flame can propagate through the vapor of a combustible material to its liquid surface. A chemical's flash point is determined by the vapor pressure of the liquid. Only when a sufficiently high concentration is reached can a solvent vapor support combustion.

Starting the detector entails powering-on the detector and each system instrument individually, as well as the data system workstation, and starting the operating software (Empower or MassLynx).



Caution:

- To prevent flow cell breakage, do not exceed the flow cell's maximum allowable pressure of 3447 kPa (34 bar, 500 psi).
- If you have installed the large-volume flow cell on the FLR detector, do not use the standard pressure regulator shipped with the detector. That regulator's 1724 kPa (17 bar, 250 psi) set point, which exceeds the large-volume flow cell's 1000 kPa (10 bar, 145 psi) pressure limit, can damage the flow cell. Use the backpressure regulator shipped with the large-volume flow cell, which has a set point of 689 kPa (7 bar, 100 psi).

See also: *ACQUITY UPLC System Operator's Guide*.

To start the detector

1. Power-on the workstation.
2. Press the power switch on the top, left-hand side of the binary solvent manager door and sample manager door.

Results:

- Each system instrument “beeps” and runs a series of startup tests.
 - Each system instrument’s power LED shows green.
 - During initialization, each system instrument’s status LED flashes green.
 - After the instruments are successfully powered-on, all LEDs show steady green. The binary solvent manager’s flow LED and the sample manager’s run LED remain unlit.
3. Start Empower or MassLynx software.
Tip: You can monitor the ACQUITY UPLC console for messages and LED indications.
 4. Flush the system with filtered and degassed HPLC-grade methanol or acetonitrile.
 5. Pump mobile phase for at least 15 minutes.
 6. Ensure the detector cell is filled with solvent and free of bubbles.
Tip: The detector might not initialize correctly if the cell contains air.
 7. Press the power switch on the front panel to power-on the detector.
Result: The detector runs a series of startup diagnostic tests.
Tip: The lamp LED blinks green during startup diagnostic tests but shows steady green when the lamp is ignited.
 8. When the lamp LED is steady green, start Empower or MassLynx software, and download an instrument or inlet method.
Tip: You can monitor the ACQUITY UPLC console for messages and visual signals.
Recommendation: For best results, wait 1 hour for the detector to stabilize before acquiring data.

Monitoring detector LEDs

Light emitting diodes on the detector indicate its state of functioning.

Power LED

The power LED, to the left of the detector's front panel, indicates when the detector is powered-on or powered-off.

Lamp LED

The lamp LED, to the right of the power LED, indicates the lamp status.

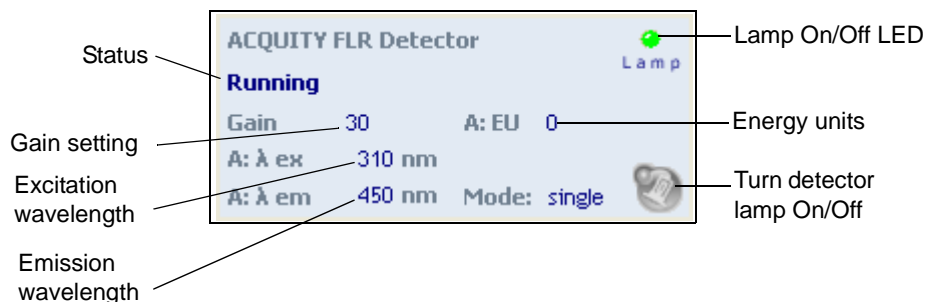
Lamp LED indications

LED mode and color	Description
Unlit	Indicates the detector lamp is extinguished.
Constant green	Indicates the detector lamp is ignited.
Flashing green	Indicates the detector is initializing or calibrating.
Flashing red	Indicates that an error stopped the detector. Refer to the console for information regarding the error.
Constant red	Indicates a detector failure that prevents further operation. Power-off the detector, and then power-on. If the LED is still steady red, contact your Waters service representative.

The detector control panel

If Empower software controls the system, the detector's control panel appears at the bottom of the Run Samples window. If MassLynx software controls the system, the detector's control panel appears at the bottom of the Inlet Editor window.



Detector control panel



The detector control panel displays the acquisition status and shutter position. You cannot edit detector parameters while the system is processing samples.

The following table lists the items in the detector control panel.

Modifiable detector control panel items

Control panel item	Description
Lamp On/Off LED	Mimics the actual lamp on/off LED mode unless communications with the detector are lost. Clicking it opens the lamp control window.
Status	Displays the status of the current operation.
Gain	Displays the PMT gain setting.
A: λ ex	Displays the excitation wavelength.
A: λ em	Displays the emission wavelength.
A: EU	Displays the energy units.
Mode	Displays the mode in which the detector is operating (single channel, multichannel, spectrum scanning, 3D mode, and lambda-lambda mode).
 (Lamp On)	Ignites the detector lamp.
 (Lamp Off)	Extinguishes the detector lamp.

You can access additional functions by right-clicking anywhere in the detector control panel:

Additional functions in the detector control panel

Control panel function	Description
Autozero	Resets the detector offsets.
Reset FLR	Resets the detector, when present, after an error condition.
Help	Displays the console Help.

Performing a run

This section explains how to perform a run using a fluorescence detector.

Before you begin this procedure, your detector must be set up and configured as described in [Chapter 2](#) and this chapter.

Preparing for the run

Preparation is the same whether the detector is controlled by the Empower or MassLynx data system.

To prepare for a run



Warning: Always observe Good Laboratory Practices when you use this equipment and when you work with solvents and test solutions. Know the chemical and physical properties of the solvents and test solutions you use. See the Material Safety Data Sheet for each solvent and test solution in use.

1. Prepare a mobile phase of 90:10 HPLC-grade water/HPLC-grade acetonitrile and label it “A”.
2. Submerge line A1 in the reservoir bottle labeled “A”.
3. Prepare a mobile phase of 100% HPLC-grade acetonitrile and label it “B”.
4. Submerge line B1 in the reservoir bottle labeled “B”.
5. Prime the ACQUITY system.

6. After the ACQUITY system has been primed, equilibrate the ACQUITY UPLC 2.1 × 50 mm BEH C18 column with 30:70 A/B.

Creating the test method

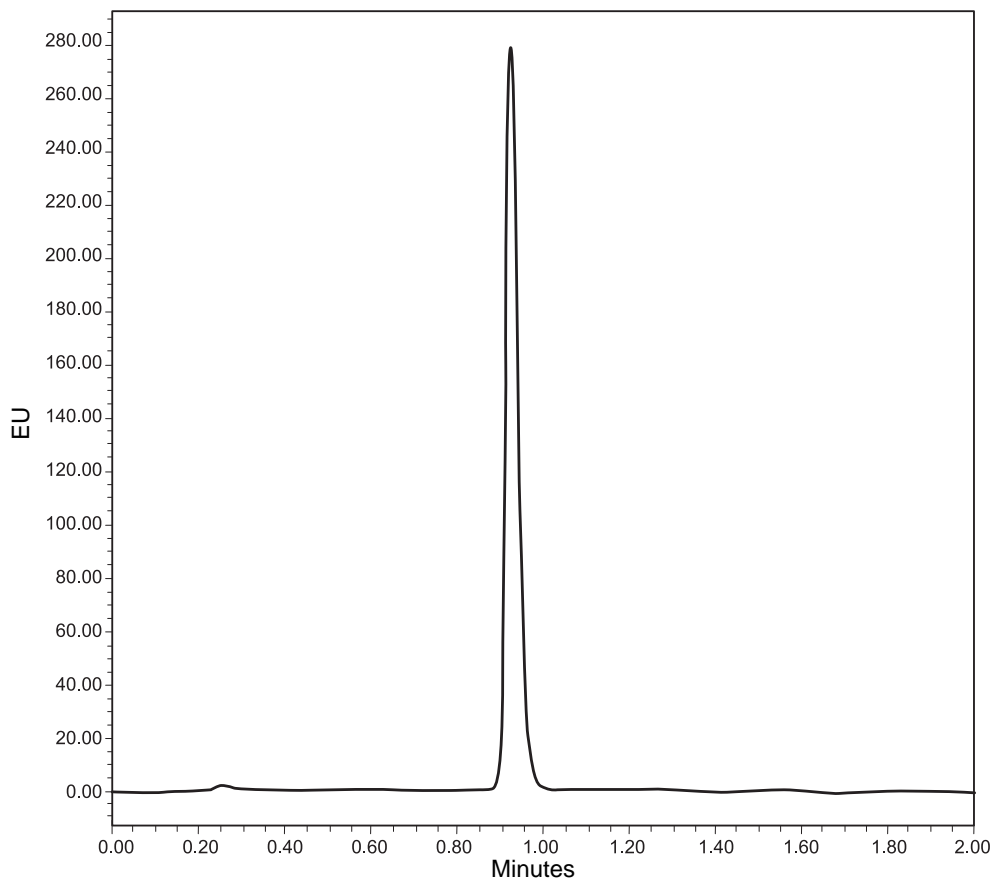
The sample used in this experiment is anthracene, 5 pg/uL, which is available as a service spare part.

Set up an isocratic run at 30:70 A/B for 2 minutes with these parameters:

- Flow rate: 0.4 mL/min
- Injection volume: 5 µL
- Excitation wavelength: 246 nm
- Emission wavelength: 402 nm
- Gain: 1
- Data rate: 10
- Time constant: Fast

The following figure shows a representative FLR detector chromatogram of one of the solutions detailed above.

FLR detector chromatogram




The peak eluting at 0.90 minutes is anthracene.

Installing the cuvette cell

To determine optimized values, use the cuvette cell to scan the excitation and emission wavelength ranges and then view the spectrum plot in the ACQUITY Console or in Empower software using the Spectrum λ - λ plot function.

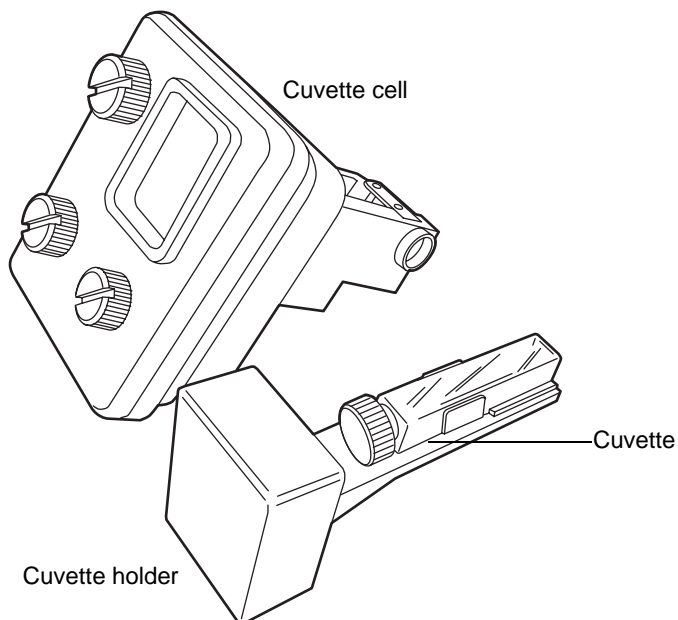
To install the cuvette cell

1. Set the gain on the FLR detector to 0.

 **Caution:** Extended exposure of the photomultiplier tube (PMT) to light can permanently damage the PMT, especially at high gain settings.

- a. In the ACQUITY UPLC console, navigate to the interactive display.
 - b. Select the control icon.
 - c. When the gain setting link turns blue, click on the value.
 - d. Specify “0” and then press “Enter.”
2. Remove the flow cell. (See [“Replacing the flow cell” on page 4-13.](#))
 3. Install the cuvette cell.

Cuvette cell, cuvette holder, and cuvette



Shutting down the detector



Caution: Buffers left in the system can precipitate and damage instrument components, including the flow cell.

Shut down the detector

- between analyses.
- overnight.
- for a weekend.
- for 72 hours or more.

Tip: If Empower software controls the system, set system shutdown parameters in the Instrument Method Editor. Consult the Empower online Help or the ACQUITY UPLC console online Help for more information.

If MassLynx software controls the system, set system shutdown parameters in the Shutdown Editor. Consult the MassLynx online Help for more information.

Between analyses

To shut down the detector between analyses

1. Continue to pump the initial mobile-phase mixture through the column to maintain the column equilibrium necessary for good retention-time reproducibility.
2. If a few hours will pass before the next injection, slow the flow rate in the interim to a few tenths of a mL/min to conserve solvent.

Tip: Ensure that the shutdown method is deactivated.

3. Keep the detector operating and the column heater at operating temperature during this period.

Shutting down for fewer than 72 hours

To shut down the detector for fewer than 72 hours

1. Flush the column with 90:10 HPLC-quality water:methanol to keep the column bed in an active, wetted state.

Requirement: If you are using buffers, you must first flush the column with a high-water-content mobile phase (90% water). Then stop the pump flow.

2. If possible, extinguish the detector lamp to lengthen lamp life.

Recommendation: The column heater can operate overnight but shut it down over a weekend.

Shutting down for more than 72 hours

To shut down the detector for more than 72 hours

1. Follow the steps for shutting down the detector for fewer than 72 hours, above.
2. After flushing the column and letting it cool to ambient temperature, disconnect the inlet and outlet tubes, and join them with a union.
3. Install end-plugs in the column inlet and outlet fittings, and then return the column, carefully, to its box for storage.



Caution:

- If any system instruments are to be used for another type of analysis, ensure that the liquids pumped initially through the system are miscible with methanol, water, methanol/acetonitrile, or isopropyl alcohol. Likewise, before restarting the system, ensure that any residual material not miscible with the initial methanol/water mobile phase has been flushed thoroughly from the system with an appropriate intermediate solvent.
 - If the flow cell will not be used for a period of time, flush it with clean mobile phase, such as a water/acetonitrile or water/methanol mix, and either cap the flow ports or dry the flow cell with pure lab gases such as helium, nitrogen, or air for 5 to 10 minutes.
4. Pump water through the system for 10 to 20 minutes at 0.5 mL/min.
 5. Pump isopropyl alcohol through the system for 10 to 20 minutes.
 6. Turn off the pump, leaving the alcohol in the fluid lines.

4

Maintaining the Detector

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Contacting Waters technical service

If you are located in the USA or Canada, report malfunctions or other problems to Waters Technical Service (800 252-4752). Otherwise, phone the Waters corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. Our Web site includes phone numbers and e-mail addresses for Waters locations worldwide. Go to www.waters.com, and click Waters Division.

When you contact Waters, be prepared to provide this information:

- Error message (if any)
- Nature of the symptom
- Instrument serial number
- Flow rate
- Operating pressure
- Solvent(s)
- Detector settings
- Type and serial number of column(s)
- Sample type
- Empower or MassLynx software version and serial number
- Data system workstation model and operating system version

For complete information on reporting shipping damages and submitting claims, see *Waters Licenses, Warranties, and Support Services*.

Maintenance considerations

Safety and handling

Observe these warning and caution advisories when you perform maintenance on your detector.



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the system. 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 electric shock:

- Do not open the detector cover. The detector does not contain user-serviceable components.
- Power-off and unplug the detector before performing any maintenance on the instrument.



Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the detector. To interrupt power to the detector, set the power switch to Off, and then unplug the power cord from the AC outlet. Afterward, wait 10 seconds before you disconnect an assembly.

Proper operating procedures

To ensure your system runs efficiently, follow the operating procedures and guidelines in [Chapter 3](#).

Spare parts

Replace only parts mentioned in this document. For spare parts details, see the Waters Quality Parts Locator on the Waters Web site's Services & Support page.

Recommendations:

- Power-off the detector at least every 2 weeks to initiate verification.
- To prevent dirt from getting into the optics assembly, always keep the detector door closed whenever a flow cell is not installed in the detector.
- Filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.
- If the lamp will not be in use for 4 or more hours, extinguish it. Leave the detector running but idle.



Caution: To avoid damaging the detector or column, remove the column and disconnect the detector before you flush the system.

Routine maintenance

The detector requires minimal routine maintenance:

- Replace solvent reservoir filters regularly.
- Filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.
- Flush buffered mobile phases out of the detector with HPLC-grade water followed by a 5 to 10% methanol solution each time the detector is shut down. This prevents
 - plugging of the solvent lines and flow cell.
 - damage to the components.
 - microbial growth.

Maintaining the leak sensor

A leak sensor in the FLR drip tray continuously monitors the detector for leaks. The sensor stops system flow when it detects accumulated, leaked liquid in its surrounding reservoir, and an error message describing the problem appears in the ACQUITY UPLC Console.

Resolving detector leak sensor errors

After approximately 1.5 mL of liquid accumulates in the leak sensor reservoir, an alarm sounds, indicating that the leak sensor detected a leak.



Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Caution: To avoid scratching or damaging the leak sensor

- do not allow buffered solvents to accumulate and dry on it.
- do not submerge it in a cleaning bath.

Required materials

- Clean, chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

To resolve a detector leak sensor error

1. View the Leak Sensors dialog box in the ACQUITY UPLC Console to confirm that the detector leak sensor detected a leak.

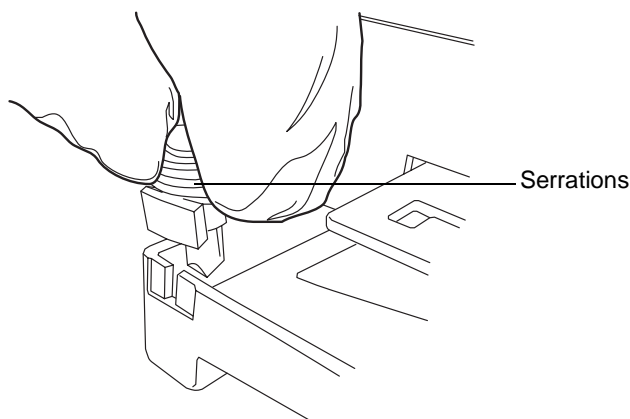
Tip: If a leak is detected, a “Leak Detected” error message appears.

2. Open the detector door, gently pulling its right-hand edge toward you.
3. Locate the source of the leak, and make the repairs necessary to stop the leak.



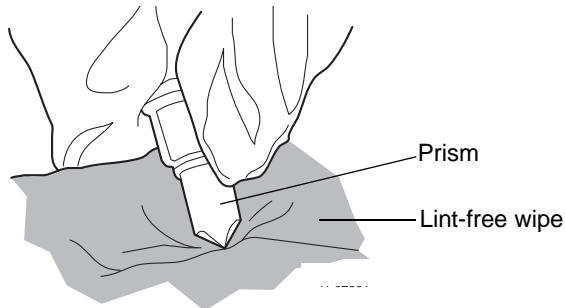
Caution: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

4. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.

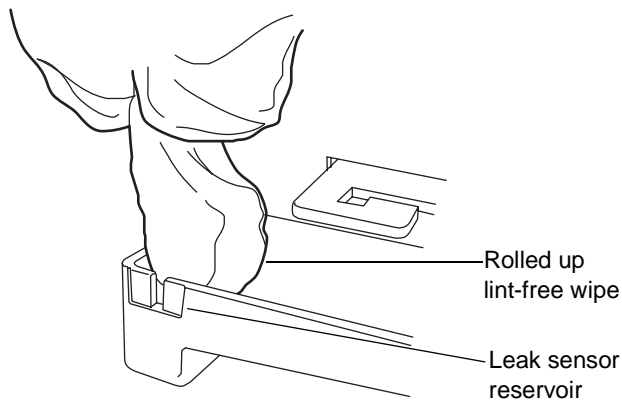


Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the instrument (see [page 4-8](#)).

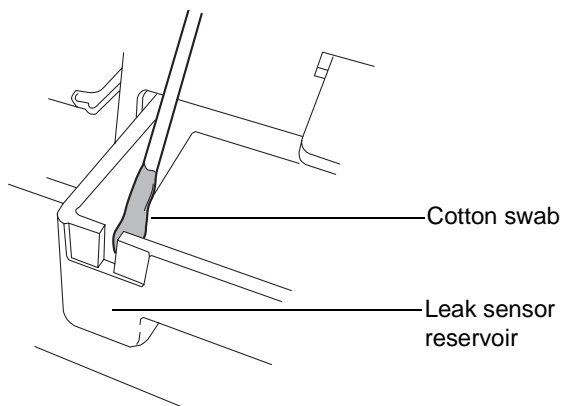
5. Use a nonabrasive, lint-free wipe to dry the leak sensor prism.



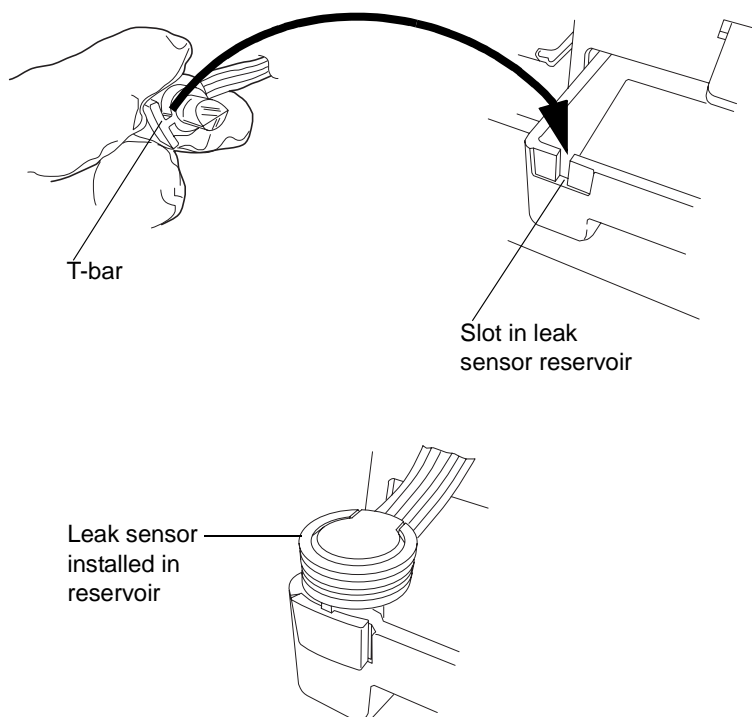
6. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak sensor reservoir and its surrounding area.



7. With a cotton swab, absorb any remaining liquid from the corners of the leak sensor reservoir and its surrounding area.



8. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



9. If you detached the connector from the front of the instrument, reattach it.
10. In the ACQUITY UPLC Console, select your detector from the system tree.
11. In the detector information window, click **Control > Reset**, to reset the detector.

Replacing the detector's leak sensor



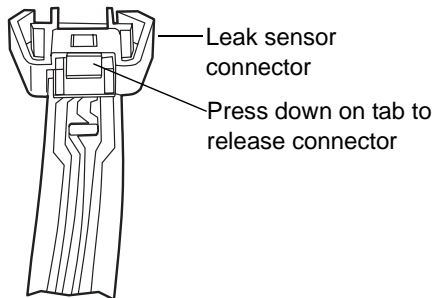
Warning: The leak sensor can be contaminated with biohazardous and/or toxic materials. Always wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required materials

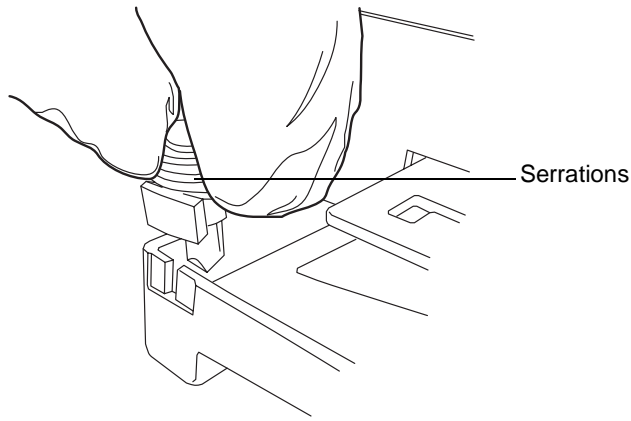
- Clean, chemical-resistant, powder-free gloves
- Leak sensor

To replace the detector leak sensor

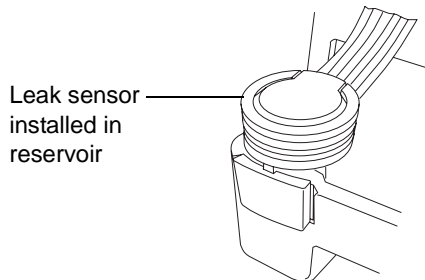
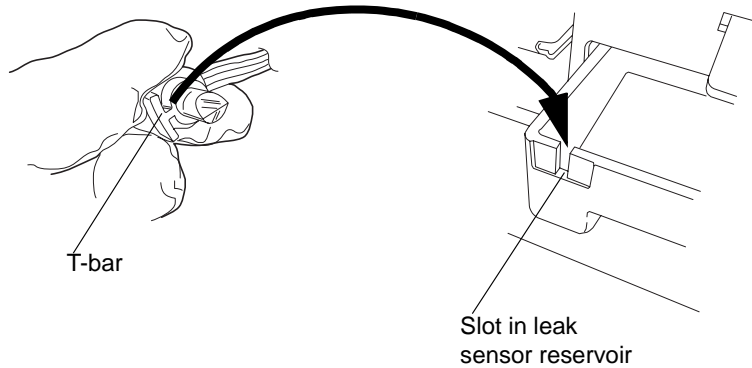
1. Open the detector door, gently pulling its right-hand edge toward you.
2. Press down on the tab to detach the leak sensor connector from the front of the instrument.



3. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.



4. Unpack the new leak sensor.
5. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and slide the leak sensor into place.



6. Plug the leak sensor connector into the front of the instrument.
7. In the ACQUITY UPLC Console, select your detector from the system tree.
8. In the detector information window, click Control > Reset, to reset the detector.

Maintaining the flow cell

Precautions

To prevent contamination, use powder-free finger cots or gloves when handling, removing, or replacing a flow cell.



Caution: To avoid damaging the flow cell,

- handle it with care.
- do not disassemble it.
- preflush columns with clean mobile phase before connecting them to the flow cell. For example, flush a 2.1 × 50 column for 10 minutes at 0.5 mL/min.

Required materials

- Wrench, suitable for removing and replacing the fittings
- Stainless steel union and tubing
- A solvent like methanol, which is miscible in both the mobile phase and water
- Powder-free finger cots or gloves
- Strong cleaning solvent suitable for your system
- HPLC-quality water
- Separate container for acid waste

Flushing the flow cell

Flush the flow cell when it becomes contaminated with the residues of previous runs and after each detector shutdown. A dirty flow cell can cause baseline noise, decreased energy levels, calibration failure, and other

problems. Always flush and purge the flow cell as your initial attempt to correct these problems.

Flush the flow cell whenever

- noise is higher than expected.
- Raman signal-to-noise test results are not meeting specifications.
- the detector fails to normalize.



Caution: To avoid damaging the flow cell during reverse flushing, do not overpressure the cell.

If you use buffered mobile phase, flush it from the detector before powering-off.




Caution:

- If the flow cell will not be used for a period of time, flush it with clean mobile phase, such as a water/acetonitrile or water/methanol, then cap the flow ports or dry the flow cell with pure nitrogen or pure helium.
- To prevent flow cell failure, do not connect any tubing or device that can create backpressure exceeding the flow cell's maximum rating of 3447 kPa (34 bar, 500 psi).
- If you have installed the large-volume flow cell on the FLR detector, do not use the standard pressure regulator shipped with the detector. That regulator's 1724 kPa (17 bar, 250 psi) set point, which exceeds the large-volume flow cell's 1000 kPa (10 bar, 145 psi) pressure limit, can damage the flow cell. Use the backpressure regulator shipped with the large-volume flow cell, which has a set point of 689 kPa (7 bar, 100 psi).


Tip: Always use well-degassed eluents.

To flush the flow cell


1. Stop the solvent flow, and remove the column.
2. Replace the column with a union or piece of tubing.

 **Caution:** If the mobile phase is not compatible with water, flush with an intermediate solvent first.

3. Flush the detector with HPLC-quality water.

 **Caution:** Do not exceed 3447 kPa (34 bar, 500 psi) with the standard cell. If you have installed the large-volume flow cell, do not exceed that flow cell's 1000 kPa (10 bar, 145 psi) pressure limit.

4. Pump 100% methanol through the flow cell to clean it internally.

 **Caution:** Do not exceed 3447 kPa (34 bar, 500 psi) with the standard cell. If you have installed the large-volume flow cell, do not exceed that flow cell's 1000 kPa (10 bar, 145 psi) pressure limit.

5. Pump a strong cleaning solvent, such as isopropanol, through the flow cell (optional).

6. If the mobile phase is miscible in water, resume pumping mobile phase. Otherwise, flush first with an intermediary solvent.

7. Reattach the column.

Recommendation: Renormalize in 100% water before resuming analyses.

8. If the flow cell remains dirty or blocked, reverse flush it.

Reverse flushing the flow cell

If directly flushing the flow cell does not improve flow cell performance, reverse flush it.

To reverse flush the flow cell

1. Reverse the inlet and outlet tubing connections to the flow cell.



Caution:

- To avoid overpressuring the standard flow cell, do not exceed 3447 kPa (34 bar, 500 psi).
 - If you have installed the large-volume flow cell on the FLR detector, do not use the standard pressure regulator shipped with the detector. That regulator's 1724 kPa (17 bar, 250 psi) set point, which exceeds the large-volume flow cell's 1000 kPa (10 bar, 145 psi) pressure limit, can damage the flow cell. Use the backpressure regulator shipped with the large-volume flow cell, which has a set point of 689 kPa (7 bar, 100 psi).
2. Flush the flow cell for approximately 15 minutes.
Tip: Decreasing system pressure indicates the flow cell is clean.
 3. If the flow cell remains dirty or blocked, remove and replace it.
 4. Return the blocked flow cell to Waters (see [“Contacting Waters technical service” on page 4-2](#)).

Replacing the flow cell

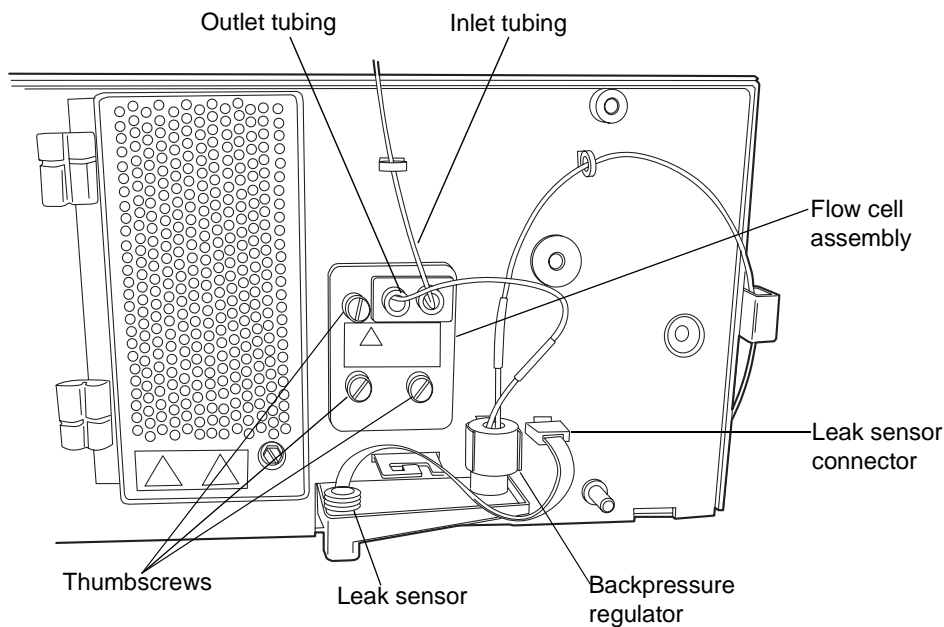
Required materials

- 1/4-inch flat-blade screwdriver
- Flow cell

To replace the flow cell

1. Power-off the detector.
2. Stop the solvent flow.
3. Open the detector door, gently pulling its right-hand edge toward you.

4. Disconnect the detector's inlet tubing from the column outlet connection.



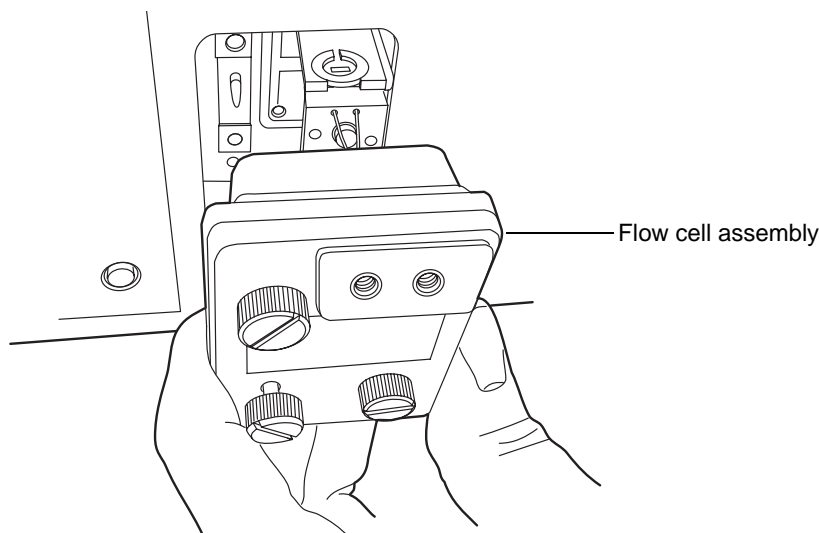
5. Disconnect the backpressure regulator, if present.



Caution: Failure to tilt the bottom of the flow cell upward can damage the flow cell mask.

6. Remove the flow cell
 - a. Loosen the 3 thumbscrews on the flow cell assembly's front plate.
 - b. Tilt the bottom of the flow cell upward, and gently pull it toward you.
7. Unpack and inspect the new flow cell.

8. Square the flow cell assembly in front of the opening, and then insert it slowly so that the pins on the front part of the flow cell compartment line up to the holes in the flow cell detector.



9. Continue to insert the flow cell until the 3 thumbscrews align with their holes in the bulkhead.
10. Finger-tighten the thumbscrews.



Caution: When installing the large-volume fluorescence flow cell on the FLR detector, do not use the standard pressure regulator shipped with the detector. That regulator's 1724 kPa (17 bar, 250 psi) set point, which exceeds the large-volume flow cell's 1000 kPa (10 bar, 145 psi) pressure limit, can damage the flow cell. Use the backpressure regulator shipped with the large-volume flow cell, which has a set point of 689 kPa (7 bar, 100 psi).

11. Connect the inlet tubing to the column outlet connection and flow cell inlet, and connect the outlet tubing to the backpressure regulator.
12. Close the detector door.
13. Before you power-on the detector, prime the system to fill the flow cell with solvent and remove any air.

Replacing the lamp

Change the lamp when it repeatedly fails to ignite or when the detector fails to calibrate.

The FLR detector source lamp is warranted to ignite and pass startup diagnostic tests for 1000 hours or 1 year from the date of purchase, whichever comes first.



Warning: The lamp housing gets extremely hot during operation. To prevent burn injuries,

- allow the lamp to cool for 60 minutes before removing it.
- keep the lamp in the housing when handling it.



Warning: The gas pressure inside the lamp increases when the lamp is hot. To avoid injury, allow the lamp to cool for 60 minutes before removing it.



Warning: To avoid eye injury from ultraviolet radiation exposure,

- wear eye protection that filters ultraviolet light.
- keep the lamp in the housing during operation.

Required materials

- Phillips screwdriver
- Flow cell

To remove the lamp module

1. Click FLR Detector in the left-hand pane of the console, and then click  to power off the lamp.

Result: The green LED on the console darkens as does the Lamp LED on the door.

2. Open the detector door, gently pulling its right-hand edge toward you.
3. To help the lamp cool more quickly, open the lamp access door with a small flat-blade screwdriver.



Warning: The lamp and its housing can be hot. Wait 60 minutes for these components to cool before touching them.

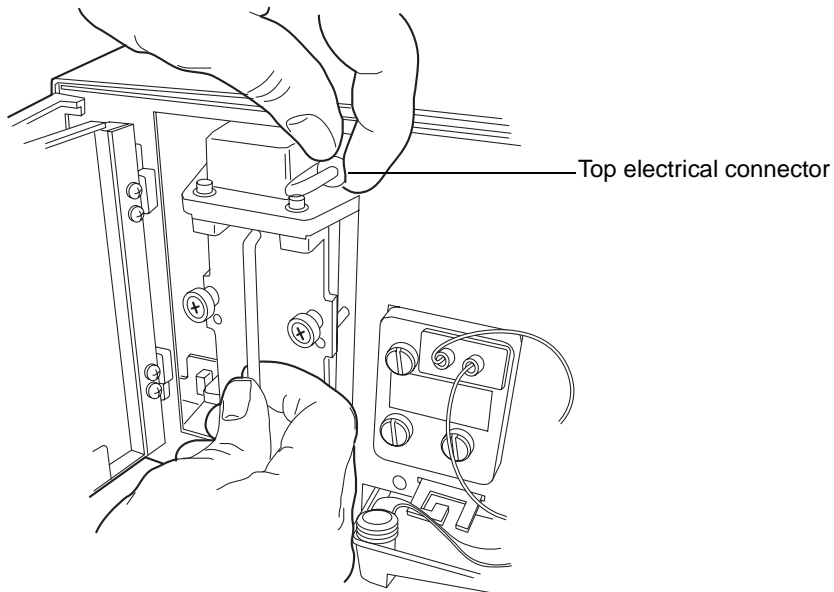
4. Allow the lamp to cool for 60 minutes.

Tip: Leaving the detector powered-on while the lamp is cooling allows the fans to keep running, which helps the lamp to cool more quickly.

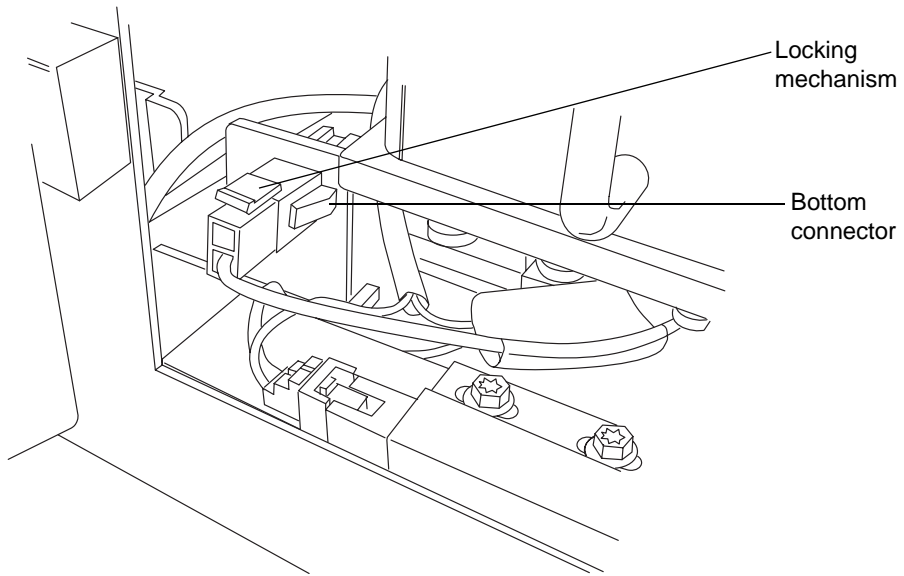


Caution: Do not grasp the connector by the wire. Doing so can damage the connector or cable.

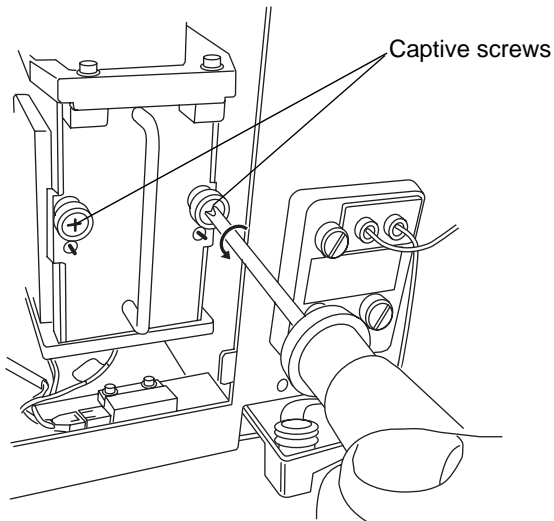
5. Power-off the detector and disconnect the power cable from the rear panel.
6. Gently pull the top electrical connector straight out.



7. Pinch the locking mechanism on the bottom connector before pulling it out.



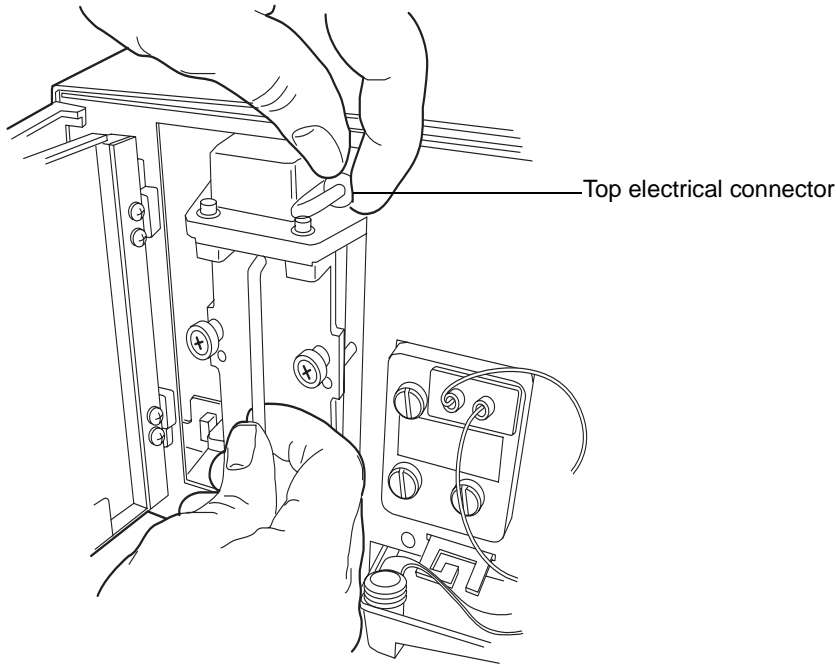
8. Using a Phillips screwdriver, loosen the 2 captive screws on the lamp housing.





Warning: To avoid injury, always keep the lamp facing away from you when removing it.

9. Gently withdraw the lamp from its housing.



Warning:

- Lamp gas is under positive pressure. To prevent shattering the glass, use care when disposing of the lamp. Waters suggests that you adequately cushion an old lamp by containing it in the packaging of its replacement before you dispose of it.
- The lamp contains mercury. Do not dispose of the lamp in municipal waste. Refer to your local environmental laws regarding disposal and recycling of lamps that contain mercury.



Caution: Do not touch the glass bulb of the new lamp. Dirt or fingerprints adversely affect detector operation. If the bulb needs cleaning, gently rub it with ethanol and lens tissue. Do not use abrasive tissue. Do not apply excessive pressure.

To install the lamp

1. Unpack the new lamp from its packing material without touching the bulb.
2. Inspect the new lamp and lamp housing.
3. Record the serial number, which is located on a label attached to the lamp connector wire.
4. Reconnect the bottom lamp power connector, ensuring that it locks into place.
5. Reconnect the top lamp power connector.
6. Position the lamp cartridge and insert it in the housing.
Tip: No additional alignment is required.
7. Push the lamp forward gently, until it bottoms into position.
8. Tighten the 2 captive screws.
9. Close the lamp access door.
10. Power-on the detector, and then wait about 30 minutes for the lamp to warm before resuming operations.
Tip: Cycling power to the detector (that is, powering-off and then powering-on the instrument) initiates the verification procedures.
11. In the console, select Maintain > Change Lamp.
12. Click New Lamp.
Tip: If you do not record a new lamp's serial number in the ACQUITY UPLC console, the date of the previous lamp installation remains in the detector's memory, voiding the new lamp's warranty.
13. Type the serial number for the new lamp (see the label attached to the lamp connector wire), and then click OK.

Replacing the fuses



Warning: To avoid electric shock, power-off and unplug the FLR detector before examining the fuses. For continued protection against fire, replace fuses with those of the same type and rating only.

The detector requires two 100 to 240 VAC, 50 to 60-Hz, F 3.15-A, 250-V (fast-blow), 5 × 20 mm (IEC) fuses.

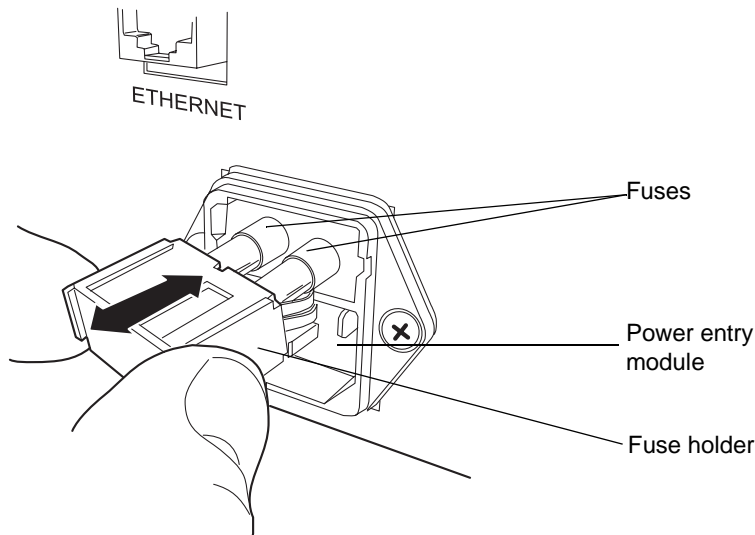
Suspect a fuse is open or otherwise defective when

- the detector fails to power-on.
- the fans do not operate.

To replace the fuses

Requirement: Replace both fuses, even when only one is open or otherwise defective.

1. Power-off the detector and disconnect the power cord from the power entry module.
2. Pinch the sides of the spring-loaded fuse holder, located above the power entry module on the rear panel of the detector.
3. With minimum pressure, withdraw the spring-loaded fuse holder.



4. Remove and discard the fuses.
5. Make sure that the new fuses are properly rated for your requirements, and then insert them into the holder and the holder into the power entry module, gently pushing until the assembly locks into position.
6. Reconnect the power cord to the power entry module.

Cleaning the instrument's exterior

Use a soft cloth, dampened with water, to clean the outside of the detector.

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
Warning symbols	A-2
Caution symbol	A-5
Warnings that apply to all Waters instruments	A-5
Electrical and handling symbols	A-12

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 needle puncture.)



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



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



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



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



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

Warnings that apply to particular instruments, instrument components, and sample types

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 Bedienungsbezugnis 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.







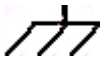
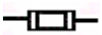



警告: 火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Electrical and handling symbols





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


ACQUITY UPLC FLR detector specifications

Tip: The specifications outlined in this appendix depend on the conditions in your laboratory. Refer to the *ACQUITY UPLC Site Preparation Guide* or contact Waters Technical Service for more information on specifications.

Environmental specifications

Attribute	Specification
Operating temperature	4 to 40 °C (39.2 to 104 °F)
Operating humidity	20 to <95%, noncondensing
Shipping and storage temperature	-30 to 60 °C (-22 to 140 °F)
Shipping and storage humidity	0 to <95%, noncondensing

Electrical specifications

Attribute	Specification
Protection class ^a	Class I
Oversvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPX0), indoors
 Line voltages, nominal	Grounded AC
Altitude	2000 m (6561.6 feet)
Pollution degree	2
Power requirements	100 to 240 VAC
Line frequency	50 to 60 Hz

Electrical specifications (Continued)

Attribute	Specification
Power consumption	280 VA (nominal)
Outputs	Four outputs (2 analog and 2 event)
Inputs	Four event inputs

- a. **Protection Class I** – The insulating scheme used in the instrument to protect from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.
- b. **Overvoltage Category II** – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.
- c. **Pollution Degree 2** – A measure of pollution on electrical circuits, which may produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally nonconductive pollution. Occasionally, however, expect a temporary conductivity caused by condensation.
- d. **Moisture Protection** – Normal (IPXO) – IPXO means that no Ingress Protection against any type of dripping or sprayed water exists. The X is a placeholder that identifies protection against dust, if applicable.

Performance specifications

Attribute	Specification
Wavelength range	200 to 890 nm (excitation) 210 to 900 nm (emission)
Bandwidth	20 nm
Wavelength accuracy	±3 nm
Wavelength repeatability	±0.25 nm
Sensitivity ^a	Signal-to-noise >1000 (Raman spectrum of water)
Measurement range	0.001 to 10,000 EU
Data acquisition	Up to 80 Hz
Unattended operation	Leak sensor, full 96-hour diagnostic data displayed through ACQUITY UPLC console software
Optical component specifications	
Light source	Hg/Xe arc lamp (1000 hour warranty)
Flow cell design	Axially illuminated

Performance specifications (Continued)

Attribute	Specification
Cell volume	<2 μ L
Pressure limit	3447 kPa (34 bar, 500 psi) for standard flow cell
Materials	Stainless steel, fused silica, FEP, PEEK™

a. Conditions: 2 Hz, 2s FTC, Ex 416 nm, EM 365 nm, single λ mode

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