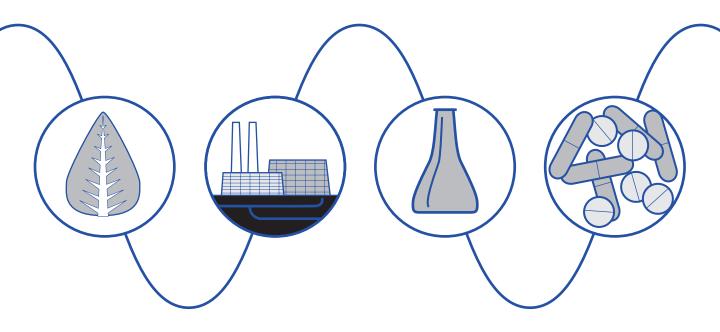
Waters 2487 Dual λ Absorbance Detector

Operator's Guide





34 Maple Street Milford, MA 01757

WAT048740, Revision 2

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Attention: The Waters 2487 Dual λ Absorbance Detector can be used for in vitro diagnostic applications. This is a highly sensitive instrument. Read the accompanying operator's guide before using.

When using the instrument, follow generally accepted procedures for quality control and methods development.

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



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.



Attention: To meet the regulatory requirements of immunity from external electrical disturbances that may affect the performance of this instrument, do not use cables longer than 9.8 feet (3 meters) when you make connections to the terminal strips on the rear panel of the unit. In addition, ensure you always connect the shield of the cable to chassis ground at one instrument only.



Caution: To protect against fire hazard, replace fuses with those of the same type and rating.



Caution: To avoid the possibility of electrical shock, power off the 2487 Detector and disconnect the power cord before you service the instrument.

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

Symbols Used on Rear Panel of the 2487 Detector

	Direct current
\sim	Alternating current
	Protective conductor terminal
<i>.</i>	Frame or chassis terminal
Â	Caution, risk of electrical shock (high voltage)
	Caution or refer to guide
	Caution, hot surface or high temperature

2487 Detector Information

Intended Use

You can use the Waters 2487 Dual λ Absorbance Detector for in-vitro diagnostic testing to analyze many compounds, including diagnostic indicators and therapeutically monitored compounds. When developing 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 covers good operating procedures and techniques necessary to validate system and method performance.

Biological Hazard

When analyzing physiological fluids, take all necessary precautions and treat all specimens as potentially infectious. Precautions are outlined in the "CDC Guidelines on Specimen Handling,"

CDC–NIH Manual, 1984.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate the standard curve. Concentration range should cover the entire range of quality control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples and monitor them daily. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of specification may not be valid. Do not report the data until you ensure that chromatographic system performance is acceptable. Conduct a failure investigation to determine the cause(s) for the out-of-specification results.

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How to Use This Guide

Purpose of This Guide

The Waters 2487 Dual λ Absorbance Detector Operator's Guide describes the procedures for unpacking, installing, operating, verifying, maintaining, and troubleshooting the Waters[®] 2487 Dual λ Absorbance Detector. It also includes appendixes describing instrument specifications, spare parts, warranty and service information, and solvent considerations.

Audience

This guide is for use by anyone who installs, maintains, operates, or troubleshoots the Waters 2487 Dual λ Absorbance Detector. All personnel who use this guide should be familiar with HPLC terms and practices, and be capable of basic HPLC system operations such as making fluidic connections.

Structure of This Guide

The Waters 2487 Dual λ Absorbance Detector Operator's Guide is divided into chapters and appendixes. Each page is marked with a tab and a footer to help you access information.

The following table defines each chapter and appendix in this guide.

Chapter/Appendix	Description
Chapter 1, 2487 Detector Theory of Operation	Summarizes the features of the Waters 2487 Detector and describes the theory and principles of operation.
Chapter 2, Installing the 2487 Detector	Describes how to install the Waters 2487 Detector, make fluidic and power connections, and connect the detector to other devices.
Chapter 3, Using the 2487 Detector	Describes how to operate the Waters 2487 Detector from power-on to power-off, and use the operator interface.
Chapter 4, Maintenance Procedures	Describes how to clean and replace various parts of the Waters 2487 Detector.

Chapter/Appendix	Description
<u>Chapter 5, Error Messages,</u> <u>Diagnostics, and</u> <u>Troubleshooting</u>	Describes the error messages, diagnostics, and recommended actions used in troubleshooting the Waters 2487 Detector.
Appendix A, 2487 Detector Specifications	Contains operational, optical, optional flow cell, and voltage specifications for the Waters 2487 Detector.
Appendix B, Spare Parts	Lists recommended and optional spare parts for the Waters 2487 Detector.
Appendix C, Warranty Information	Includes warranty, serial number, and service information for the Waters 2487 Detector.
Appendix D, Solvent Considerations	Provides information about the solvents used with Waters detectors.

Related Documents

The following table lists other documents related to the operation of the Waters 2487 Dual λ Absorbance Detector.

Title	Description
Waters 2690 Separations Module Operator's Guide	Describes the procedures for unpacking, installing, using, maintaining, and troubleshooting the Waters 2690 Separations Module.
Waters 600E Multisolvent Delivery System User's Guide	Describes the procedures for installing, using, maintaining, and troubleshooting the Waters 600E Multisolvent Delivery System.
Waters Bus SAT/IN Module Installation Guide	Provides the procedures for installing the Waters busSAT/IN Module.
Waters Alliance Dissolution System Operator's Guide	Describes the procedures for unpacking, installing, using, maintaining, and troubleshooting the Waters Alliance Dissolution System.
Waters 2790 Separations Module Operator's Guide	Describes the procedures for installing, using, maintaining, and troubleshooting the Waters 2790 Separations Module.

Related Documentation

Online Documentation

The following table describes the online documentation that supports the Millennium³² software.

Title	Description
Millennium ³² Online Help	Describes all Millennium ³² windows, menus, menu selections, and dialog boxes for the base software and all the software options. Also includes reference information and procedures for performing all tasks required to use the Millennium ³² software. Included as part of the Millennium ³² software.
Millennium ³² Read Me File	Describes product features and enhancements, helpful tips, installation and/or configuration considerations, and changes since the previous Millennium software version.
Millennium ³² LIMS Online Help	Describes how to use the Millennium ³² LIMS Interface to export results and import worklists.
Millennium ³² Toolkit Professional Online Help	Describes how to use the common object model, message-based protocol to communicate with the Millennium ³² software from a third-party application.
Millennium ³² WebServer Online Help	Describes how to use the Millennium ³² WebServer application.

Printed Base Documentation

The following table describes the printed documents that make up the $Millennium^{32}$ software base documentation

Title	Description
Millennium ³² Read Me First	Describes the Millennium ³² software documentation, software license, hardware warranty, support, and training.
<i>Millennium³² Software</i> <i>Getting Started Guide</i>	Provides an introduction to the Millennium ³² system. Describes the basics of how to use Millennium ³² software to acquire data, develop a processing method, review results, and print a report. Also covers basic information for managing projects and configuring systems.
Millennium ³² Software Data Acquisition/Processing Theory Guide	Provides theories pertaining to data acquisition, peak detection and integration, and quantitation of sample components.
Millennium ³² System Installation/Configuration Guide	Describes Millennium ³² software installation, including the stand-alone workstation, PowerStation™ system, and client/server system. Discusses how to configure the computer and chromatographic instruments as part of the Millennium ³² system. Also covers the installation, configuration, and use of acquisition servers such as the LAC/E ³² module, the busLAC/E TM card, and interface cards used to communicate with serial instruments.
Millennium ³² Release Notes	Contains last-minute information about the product. Also provides supplementary information about specific Millennium ³² software releases.

Printed Options Documentation

The following table describes the printed documents that support Millennium³² software options.

Title	Description
Millennium ³² System Suitability Quick Reference Guide	Describes the basics of the Millennium ³² System Suitability software application and describes the equations used by the System Suitability software.
Millennium ³² PDA Software Getting Started Guide	Describes the basics of how to use the Millennium ³² PDA option software to develop a PDA processing method and to review PDA results.
Millennium ³² GPC Software Getting Started Guide	Describes how to use the Millennium ³² GPC software option to develop a GPC processing method and to review GPC results.
Millennium ³² GPCV Software Getting Started Guide	Describes how to use the Millennium ³² GPCV software option to develop a GPCV processing method and to review GPCV results.
Millennium ³² Pattern Matching Software Getting Started Guide	Describes how to use the Chromatographic Pattern Matching software option to develop a pattern matching processing method and to review pattern match results.
Millennium ³² Dissolution Software Quick Start Guide	Describes how to operate the Alliance Dissolution System using Millennium ³² software.
Millennium ³² Tablet Processing Software User's Guide	Describes how to operate the Millennium ³² Tablet Processing Software with a Source for Automation Solid Dosage Assay System.
Integrity System Getting Started Guide	Describes the features of the Waters [®] Integrity System and provides step-by-step tutorials that guide a user through the use of the Millennium ³² Mass Spectrometry (MS) software option.
Millennium ³² Toolkit Programmer's Reference Guide	Describes how to use the common-object-model, message-based protocol to communicate with the Millennium ³² software from a third-party application.

Title	Description
Millennium ³² Alpha/Intel Client/Server System Administrator's Guide for Microsoft [®] Windows NT [®]	Describes how to administer Millennium ³² software client/server tasks in an NT Alpha or Intel Client/Server system.
Millennium ³² WebServer Installation/Configuration Guide	Describes how to install and configure the Millennium ³² WebServer.

Related Adobe[™] Acrobat Reader Documentation

For detailed information about using the Adobe Acrobat Reader, refer to the *Adobe Acrobat Reader Online Guide*. This Online Guide covers procedures such as viewing, navigating and printing electronic documentation from Adobe Acrobat Reader.

Printing From This Electronic Document

Adobe Acrobat Reader lets you easily print pages, pages ranges, or the entire electronic document by selecting **Print** from the File menu. For optimum print quantity, Waters recommends that you specify a Postscript printer driver for your printer. Ideally, use a printer that supports 600 dpi print resolution.

Conventions Used in This Guide

This guide uses the following conventions to make text easier to understand.

• Purple Text indicates user action. For example:

Press 0, then press Enter for the remaining fields.

• *Italic* text denotes new or important words, and is also used for emphasis. For example:

An instrument method tells the software how to acquire data.

• <u>Underlined, Blue Color</u> text indicates hypertext cross-references to a specific chapter, section, subsection, or sidehead. Clicking this topic using the hand symbol automatically brings you to this topic within the electronic document. Right-clicking and selecting **Go Back** from the popup context menu brings you back to the originating topic. For example:

Before completing the column connections, Waters recommends you perform the verification procedures described in <u>Section 3.3.2</u>, <u>Verifying the Detector</u>.

Notes, Attentions, and Cautions

• Notes call out information that is important to the operator. For example:

Note: Record your results before you proceed to the next step.

• Attentions provide information about preventing possible damage to the system or equipment. For example:



Attention: To avoid damaging the detector flow cell, do not touch the flow cell window.

• Cautions provide information essential to the safety of the operator. For example:



Caution: To avoid chemical or electrical hazards, always observe safe laboratory practices when operating the system.



Caution: To avoid the possibility of electrical shock and possible injury, always turn off the detector and unplug the power cord before performing maintenance procedures.



Caution: To avoid the possibility of burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.

1 2487 Detector Theory of Operation

This chapter introduces you to the Waters[®] 2487 Dual λ Absorbance Detector. It summarizes the 2487 Detector's features and describes the theory and principles of operation.

See <u>Appendix A, 2487 Detector Specifications</u>, for system specifications. <u>Appendix D,</u> <u>Solvent Considerations</u>, has information on high-performance liquid chromatography (HPLC) solvent considerations.

1.1 2487 Detector Description

The Waters 2487 Dual λ Absorbance Detector (<u>Figure 1-1</u>) is a two-channel, tunable, ultraviolet/visible (UV/Vis) detector designed for high-performance liquid chromatography (HPLC) applications.

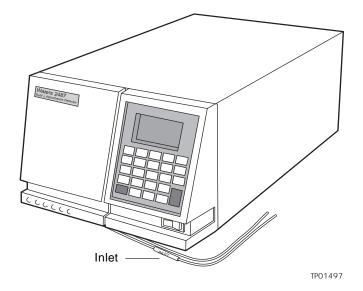


Figure 1-1 Waters 2487 Dual λ Absorbance Detector

The Waters 2487 Dual λ Absorbance Detector can operate either as a stand-alone unit (with a chart recorder or integrator) or as an integral part of a Waters chromatography system.

The Waters 2487 Dual λ Absorbance Detector can be configured with the following Waters systems:

- Millennium®³² Chromatography Manager
- Waters Alliance[®] HPLC systems (which could be the 2690, 2690D, or 2790 Separations Module)
- Waters PowerLine[™] system

Note: When the 2487 Detector is operating in dual wavelength mode and connected to Millennium³² software versions lower than 3.0, PowerLine, or a Waters 2690 Alliance Separations Module version 1.15 or lower using the IEEE-488 communications bus, you must enable the **Emulate dual 486** configuration option.

Features

The Waters 2487 Dual λ Absorbance Detector operates from 190 to 700 nm. The 2487 Detector uses newly designed optics with an enhanced illumination system for improved performance. These design features increase the optical throughput and sensitivity, and reduce bandwidth. This results in an overall increase in signal-to-noise ratio and improved linearity.

The Waters 2487 Dual λ Absorbance Detector has the following capabilities:

- Stand-alone programmability Stores up to 10 user-defined programs (or *methods*) consisting of up to 16 programmable timed events and 2 threshold events each.
- Single or dual wavelength Monitors absorbance at one or two discrete wavelengths.
- Wavelength verification reference Ensures wavelength accuracy.
- Automatic second order filter Automatically engaged for wavelengths 370 nm and greater and removed for wavelengths 369 nm or less.
- Spectrum scan and storage Supports spectrum scan, display, subtraction, storage, and playback, in addition to standard tunable absorbance and UV/Vis functionality.
- Cuvette qualification Facilitates qualification of the detector by insertion of a standard in a cuvette without breaking any fluidic connections. Waters qualification kits are available in cuvette form to support this feature. This feature also allows you to use the 2487 Detector as a benchtop spectrophotometer.
- Cuvette sample analysis Allows the spectrum of any sample placed in the cuvette to be recorded.

- Method editing and storage Supports basic method programming, storage, and retrieval from the front panel.
- Full diagnostic capability Supports built-in diagnostic tools to optimize functionality and performance.
- Backward compatibility Operates as a 486 Detector with PowerLine, Millennium³² software versions lower than 3.0, and other Waters products connected via the LAC/E[™] interface using the IEEE-488 bus.
- Two contact closure outputs The 2487 Detector has two configurable switches, each of which can accommodate a maximum of <u>+</u>30 Vdc, 1.2 A current carry, and 0.5 A current switching. The switches (SW1 and SW2) can trigger fraction collectors and other external devices, as well as activate based on time, absorbance threshold, or ratio criteria.

1.2 Principles of Operation

To use the Waters 2487 Dual λ Absorbance Detector effectively, you should be familiar with the optical and electronic design of the detector and the theory and principles of operation.

This section describes the following parts and functions of the Waters 2487 Dual λ Absorbance Detector:

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

1.2.1 Waters 2487 Detector Optics

The Waters 2487 Dual λ Absorbance Detector optics are based on a Fastie Ebert tunable monochromator and include the following:

- Deuterium (D₂) lamp
- Two mirrors: one off-axis ellipsoidal mirror and one spherical mirror
- · Shutter, wavelength calibration filter, and second order filter
- Entrance slit
- Blazed, plane holographic diffraction grating
- Beamsplitter
- Sample and reference photodiodes

- Waters TaperSlit[™] Flow Cell (its entrance is the exit slit of the monochromator)
- · Cuvette holder

Figure 1-2 shows a diagram of the 2487 Detector optics assembly light path and components.

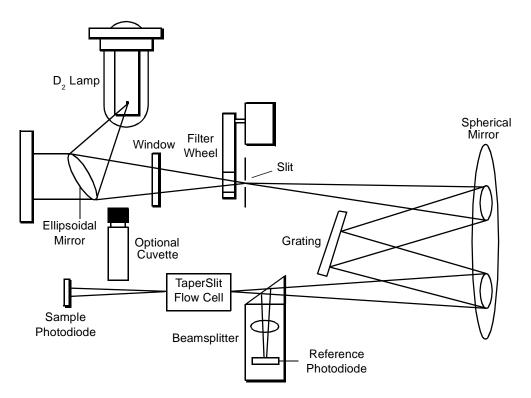


Figure 1-2 Waters 2487 Dual λ Absorbance Detector Optics Assembly

Optics Assembly Light Path

The Waters 2487 Dual λ Absorbance Detector provides an extremely efficient design for exceptionally high light throughput. It operates as follows:

 The ellipsoidal mirror collects light from the lamp and focuses it through the filter wheel onto the entrance slit. The spherical mirror directs light toward the grating. A different portion of the spherical mirror focuses dispersed light of a particular wavelength, determined by the grating angle, onto the entrance of the flow cell. Light exiting the flow cell passes through the cuvette location to the sample photodiode.

- 2. The beamsplitter, located just ahead of the flow cell, diverts a portion of the light to a reference photodiode.
- 3. When you enter a new wavelength through the 2487 Detector's front panel (or through the Millennium³²software or other system software control), the detector rotates the grating to the appropriate position.
- 4. The preamplifier board integrates and digitizes the currents from the photodiodes for processing by the signal processing electronics and output to a computer, chart recorder, or integrator.

1.2.2 Wavelength Verification and Test

The 2487 Detector deuterium arc lamp and the integral erbium filter exhibit peaks in the transmission spectrum at known wavelengths.

Upon startup, the 2487 Detector verifies calibration by comparing the locations of these peaks with expected wavelengths based on calibration data stored in the detector's memory. If the results of this verification differ from the stored calibration by more than 1.0 nm, the detector displays a Wavelength Verification Failure message. The 2487 Detector verifies rather than recalibrates on startup to avoid errors that may occur because of residual materials left in the flow cell and/or the cuvette.

Note: Always ensure that the cuvette is removed and the cuvette holder and left front panel door are secured during startup verification.

You may initiate a manual wavelength calibration at any time. A manual calibration replaces the previous calibration data with new data. See <u>Section 3.3.3, Wavelength</u> <u>Calibration</u>, for the manual wavelength calibration procedure.

The verification and calibration algorithms are virtually identical. However, the verification algorithm may issue an error message indicating that actual data does not match stored data, where the calibration algorithm replaces the stored data with the new data.

The 2487 Detector wavelength verification procedures establish an approximate home position using a grating homing sensor. Once home is established, the 2487 Detector locates and references the 656.1 nm peak in the deuterium lamp emission spectrum.

The integral erbium filter moves into the common light path ahead of the flow cell entrance slit, enabling the 2487 Detector to locate three additional spectral features at these wavelengths:

- 256.7 nm (UV)
- 379.0 nm
- 521.5 nm

The verification tests for the 2487 Detector require 5 minutes of lamp warmup time so the lamp can stabilize.

When the 2487 Detector is run continuously, we recommend you perform wavelength verification weekly by turning the detector off, then on again. See also <u>Section 3.3.3</u>, <u>Wavelength Calibration</u>.

1.2.3 Waters TaperSlit Flow Cell

The Waters TaperSlit Flow Cell (U.S. Patent No. 5,883721, March 16, 1999) used in the 2487 Detector renders the detector baseline insensitive to changes in mobile phase refractive index (RI). RI changes occur during gradient separations or result from temperature or pump-induced pressure fluctuations.

To achieve RI immunity, a combination of a spherical mirror, a lens at the entrance of the flow cell, and a taper to the internal bore of the flow cell prevents light rays from striking the internal walls of the flow cell. An additional feature of the Waters TaperSlit Flow Cell and the reason for its name is the shape of the flow cell entrance, which matches the shape of the entrance slit. Compared to a conventional flow cell with a circular entrance, the 2487 Detector achieves higher light throughput for a given spectral resolution with the TaperSlit cell design.

As shown in Figure 1-3, in a conventional cell, light bends and hits the wall of the flow cell. Four beams go in, only two come out. In the Waters TaperSlit Analytical Cell, the combination of the lens and TaperSlit bore geometry prevents light from hitting the cell walls. Four beams go in, four beams come out.

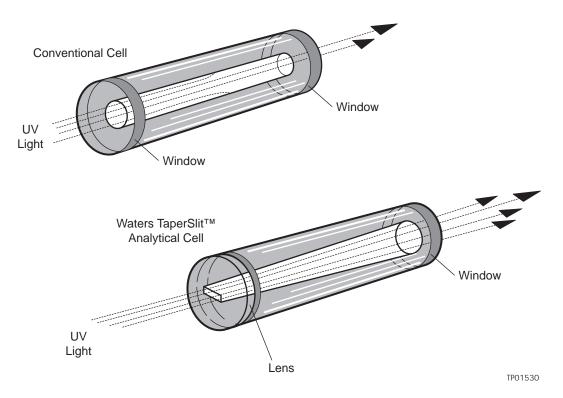


Figure 1-3 Comparison of Flow Cell Characteristics

The standard analytical, inert, and LC/MS cells have a path length of 10 mm. The semi-prep and microbore cell path length is 3 mm. A variable path length flow cell (path length 0.5 to 3 mm) is also available.

Filtering Noise

The 2487 Detector provides two filters to minimize noise:

- Hamming filter The default filter is the Hamming filter, a digital finite impulse response filter, which creates the same amount of peak height degradation as the RC filter, and also enhances the filtering of high frequency noise.
- **RC filter** The RC filter is an analog infinite impulse response filter, a first-order resistor-capacitor filter.

The behavior of the filter depends on the filter time constant that you select. The filter time constant adjusts the filter response time to achieve an optimal signal-to-noise ratio.

Lower time constant settings:

- Remove less baseline noise
- · Produce narrow peaks with minimal peak distortion and time delay
- Make very small peaks harder to discriminate from baseline noise

Higher time constant settings:

- Greatly decrease baseline noise
- · Shorten and broaden peaks

The default time constant of 1.0 second in the Hamming mode is satisfactory for most applications.

Note: For columns with an inner diameter of 2.1 mm or less, consider using the 0.3-second Hamming filter setting.

To calculate an appropriate time (filter) constant for special applications, use the formula:

$$TC = 0.2 x PW$$

where:

TC = time constant (filter) setting

PW = peak width at half height of the narrowest peak

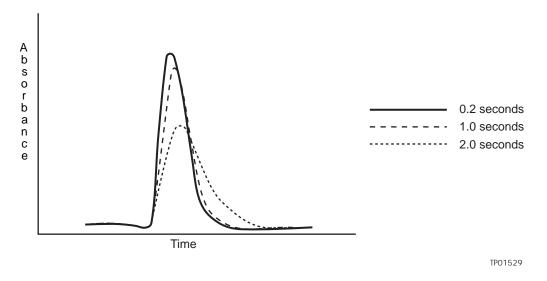


Figure 1-4 shows the relationship between increased time constant and response times.

Figure 1-4 Time Constant (Filter Setting) Comparison

Note: Although the peak shape shows some distortion and the signal output is delayed with different time constants, the peak area remains the same.

1.2.4 Waters 2487 Dual λ Absorbance Detector Electronics

The 2487 Detector electronics control the following components:

- Preamplifier board Collects and processes the analog input signals from the photodiodes to the microprocessor for further signal conditioning. Sample and reference signals are integrated and A/D conversion is performed simultaneously. This ensures the best rejection of common mode noise in the two beams, leading to a very quiet baseline.
- **Control board** Receives inputs from the preamplifier board, keyboard, and external events, and provides control of the optics positioning subsystems and lamp power supply.
- IEEE-488 interface IEEE-488 communication allows the 2487 Detector to communicate with other Waters modules that have the IEEE-488 interface capability and the appropriate software.
- **Keypad** Allows you to access the control system, program methods, calibrate, and troubleshoot the 2487 Detector.
- Lamp power supply Controlled by the CPU to provide stable deuterium lamp operation.

• **DC power supply** – Provides voltage for the analog and digital circuitry. It is the dc power source for the detector.

1.3 Operational Modes

The 2487 Detector operates in single or dual wavelength mode, allows spectrum scanning using a flow cell or a cuvette, and provides RatioPlot, difference plot, and MaxPlot functions.

1.3.1 Single Wavelength Mode

Single wavelength is the default mode of operation for the 2487 Detector. The 2487 Detector supports monitoring of a single wavelength from 190 nm to 700 nm, settable in 1-nm increments on Channel A. You can configure the analog outputs for Channel B while the detector is operating in single wavelength mode, so you can use Channel B to obtain additional information about the wavelength selected on Channel A.

In single wavelength mode, the 2487 Detector automatically engages the second order filter for wavelengths 370 nm and above and removes it for wavelengths under 370 nm. The second order filter is an optical filter that blocks unwanted ultraviolet (UV) light produced by the diffraction grating that can interfere with absorbance detection above 370 nm.

You can configure several additional parameters when using the 2487 Detector in single wavelength mode.

Primary Parameters

The following are the major parameters you can use in single wavelength mode:

- Wavelength in nm You can specify a wavelength for Channel A from 190 to 700 nm settable in 1-nm increments.
- Chart type selection In single wavelength mode, you are limited to the following selections:

- Absorbance: The detector scales absorbance in real time and sends it out over the analog output. Scaling depends on the AUFS setting and the optional chart zero parameter.

– Log absorbance: The detector can plot the Log (base 10) of absorbance allowing both small and large peaks to be viewed on one chromatogram.

- **Sample energy**: You can select the sample energy for charting out the analog output. Scaling for sample energy is fixed at 150 nA/V.

- **Reference energy**: You can select reference energy for charting out the analog output. Scaling for reference energy is fixed at 150 nA/V.

 Sensitivity in AUFS – This value specifies the scaling factor for the analog output channels and corresponds to the AU value where the analog outputs saturate at full scale values. AUFS varies from 0.0001 to 4.000 AU. Sensitivity is ignored if you select Log absorbance.



Attention: Changing the sensitivity (AUFS) setting affects both the 2 V and 10 mV outputs.

- Chart polarity (+ or –) You can use the polarity to reverse the polarity of the charted chromatogram. Select + for a normal chromatogram, or for an inverted chromatogram. This function changes the direction of the plot on both the 2 V and 10 mV outputs, similar to reversing the leads to an external chart recorder.
- Filter time constant in seconds You can program a filter time in seconds. Selecting 0.0 disables all filtering.

Secondary Parameters

When on the Absorbance (or HOME) screen in single wavelength mode, pressing **Next** brings you to several pages of secondary, or less frequently used, parameters. They are:

- Absorbance offset
- Autozero on inject
- Autozero on λ changes
- · Chart mark on keypad/panel active
- Autozero on keypad/panel active

<u>Section 3.2.4, Primary and Secondary Functions</u>, and <u>Table 3-3</u> explain the functions, ranges, and defaults of these parameters.

1.3.2 Dual Wavelength Mode

In dual wavelength mode, the detector can monitor two wavelengths, one on Channel A and one on Channel B. The sampling frequency is reduced from 10 to 1 Hz, limiting use of this mode to more standard chromatography where peaks span at least 20 seconds to enable full characterization of a peak. You can use dual wavelength mode to obtain additional information about an analyte by running a RatioPlot or a MaxPlot.

The 2487 Detector allows you to select any two wavelengths from 190 to 700 nm.



Attention: In dual wavelength mode:

• If both selected wavelengths are greater than 370 nm, the detector applies the second order filter to block unwanted UV light.

• If both selected wavelengths are less than or equal to 370 nm, the detector removes the second order filter.

• If the selected wavelengths bracket the 370 nm threshold, the detector does not apply the second order filter and issues a warning message that any data collected for the wavelength above 370 nm may contain inaccuracies because of possible UV light interference (second order effects).



Attention: To prevent acquisition of invalid data when operating the 2487 Detector in dual wavelength mode under the control of the Millennium³² software or a PowerLine system, you must select a data sampling rate of 1 point per second.

Chart Out Selection Modes

When operating in dual wavelength mode, the 2487 Detector offers these choices for analog output in addition to the selections offered in single wavelength mode and explained in <u>Section 1.3.1, Single Wavelength Mode</u>. The default selection for dual wavelength mode is Absorbance.

- Absorbance (A and B) This is the standard LC mode where the current absorption is scaled and sent directly out the analog output. The scaling is dependent upon the AUFS setting and the absorbance offset. The absorbance value is scaled for both the 2 V and 10 mV analog outputs. If a setting of 1 AU/V is desired, you can set an AUFS of 2.0000 for either the A or B output channels which can be controlled independently, even in single wavelength mode.
- Log Absorbance (A and B) This mode allows the Log (base 10) of absorbance to be plotted allowing both small and large peaks to be viewed on a single chromatographic output. See <u>Section 1.3.1</u>, <u>Single Wavelength Mode</u>.
- MaxPlot This mode results in the output of the larger of the two absorbance values, scaled to the selected AUFS setting. This mode is useful when looking at multiple compounds with absorbancies at two separate wavelengths with one data channel.
- RatioPlot (A/B or B/A) This mode produces the ratio of absorbance from two wavelengths. Theoretically, the ratio is constant for a pure chromatographic peak and variable for an impure peak. This results in a nonsquared response. Instead of a programmable AUFS, the 2487 Detector provides minimum and maximum ratio values which scale the ratio plot proportionally. In addition, a configurable *minimum absorbance threshold* activates ratio output scaling only when it reaches the absorbance at both wavelengths.

• Difference Plot (A-B or B-A) – This mode plots the arithmetic difference in absorbance for the two monitored wavelengths.

1.3.3 Spectrum Scanning

STOP

Attention: When the 2487 Detector is operating under the control of the MIllennium³² software, the scanning function is disabled.

You can use the 2487 Detector as a spectrophotometer to acquire spectra from either the flow cell or the cuvette. You may scan and store up to five spectra (five reference or zero scans, and five sample scans) in memory for playback or to compare with other spectra.

The major difference between the 2487 Detector and a double-beam spectrophotometer is that the 2487 Detector employs only one flow cell or cuvette, rather than a simultaneous sample and a reference pair.

Note: To prevent contamination when scanning using the cuvette, you should use a matched pair of cuvettes for the zero and sample scans.

The 2487 Detector obtains an absorbance spectrum by performing two types of scan on either the flow cell or using the cuvette (see <u>Section 1.3.4</u>, <u>Cuvette Operations</u>):

- Zero scan A zero scan characterizes the baseline absorbance spectrum of a solvent.
- Sample scan A sample scan subtracts the zero scan, so the results displayed or charted are of the sample only.

To obtain a spectrum of a sample with the 2487 Detector, you need to run a zero scan first, followed by a sample scan. Typically, the zero scan is run with pure solvent and the sample scan is of the analyte dissolved in that solvent.

Spectra can be simultaneously charted on the Channel A output, or acquired and stored in memory for later playback.

See <u>Section 3.4.7</u>, <u>Scanning Using the Cuvette</u>, and <u>Section 3.4.8</u>, <u>Scanning Using a</u> <u>Static Flow Cell</u>, for detailed procedures.

1.3.4 Cuvette Operations

The 2487 Detector cuvette option is used to measure the absorbance spectrum of a sample in a cuvette. To generate and store a spectrum, you need to acquire:

1. A zero scan, which measures the absorbance of the contents of the cuvette and flow cell over the desired wavelength range.

2. A sample (absorbance) scan, which measures the absorbance of the analyte dissolved in mobile phase.

The 2487 Detector subtracts the zero scan from the sample scan to create a sample spectrum.

Since the cuvette scan is acquired by measuring the absorbance from a light path that includes both the flow cell and the cuvette, the solvent conditions in the flow cell should be identical for both scans. For a detailed explanation of cuvette scanning, see <u>Section 3.4.7,</u> <u>Scanning Using the Cuvette</u>.

1.3.5 RatioPlot

The 2487 Detector allows ratio plotting, that is, comparing the absorbances of a compound or analyte at two different wavelengths. The RatioPlot divides absorbances at two selected wavelengths, and plots the resulting ratio on a chart recorder or data system over one output channel (Channel A). The RatioPlot is useful in detecting hidden components present within individual peaks.

The RatioPlot of a spectrally homogeneous peak appears as a rectangular wave. The RatioPlot of an impure peak appears as a distorted wave. When obtaining a RatioPlot, you must be operating the 2487 Detector in dual wavelength mode; the RatioPlot is output on the selected channel.

For an explanation of the RatioPlot procedure, see the discussion <u>"Obtaining a RatioPlot"</u> in <u>Section 3.3.5, Operating the Detector in Dual Wavelength Mode</u>.

1.3.6 MaxPlot

The 2487 Detector allows you to obtain a MaxPlot while in dual wavelength mode. The MaxPlot function monitors absorbance at two selected wavelengths and plots the maximum absorbance value for each sample component.

When obtaining a MaxPlot, you must be operating the 2487 Detector in dual wavelength mode. The MaxPlot outputs the greater of the two absorbance values on the selected channel.

For an explanation of the maxplot procedure, see the discussion "Obtaining a MaxPlot" in <u>Section 3.3.5, Operating the Detector in Dual Wavelength Mode</u>.

2 Installing the 2487 Detector

2.1 Introduction

The Waters[®] 2487 Dual λ Absorbance Detector requires connections to electrical power and to sample and waste lines to operate in any standard laboratory environment. This chapter describes how to install the 2487 Detector and connect it to the electrical supplies and to other equipment in an HPLC system.

Figure 2-1 shows the major steps in installing the 2487 Detector.

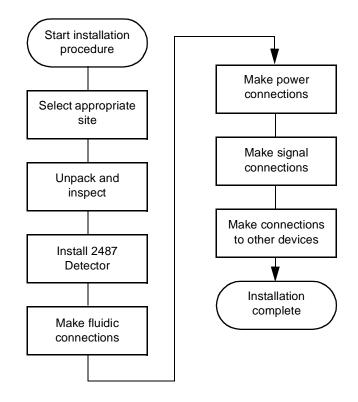


Figure 2-1 Major Steps in Installing the 2487 Detector

After installation of the 2487 Detector is complete, you should verify its functions and keep the verified chart output (if applicable) on file. Verification ensures proper operation of the detector optics and electronics. See <u>Section 3.3.2</u>, <u>Verifying the Detector</u>, for verification procedures.

Figure 2-2 illustrates the size and the physical characteristics of the Waters 2487 Dual λ Absorbance Detector.

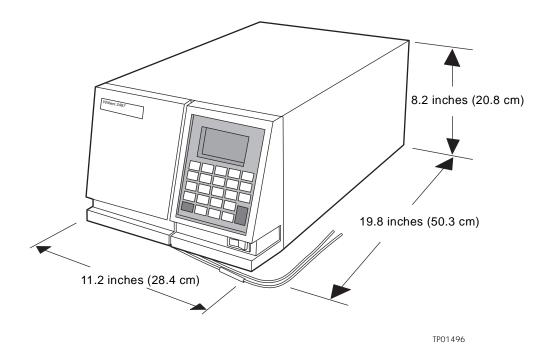


Figure 2-2 2487 Detector Dimensions



There is no access required to the instrument inside the top cover. Access is through the left front panel where the lamp housing, flow cell assembly, and cuvette holder are located.

2.2.1 Site Selection

Install the Waters 2487 Dual λ Absorbance Detector in an area that meets the requirements listed in <u>Table 2-1</u>.

Table 2-1 Installation Site Requirements

Parameter	Requirement
Operating temperature range	4 to 40 °C (39 to 104 °F)
Storage temperature range	-40 to 70 °C (-104 to 158 °F)
Relative humidity	20 to 80%, noncondensing
Storage humidity range	0 to 90%, noncondensing
Bench space	5 inches (12.7 cm) clearance at rear
Vibration	Negligible
Static electricity	Negligible
Power	Grounded ac, 100/240 Vac, 50/60 Hz

You must mount the 2487 Detector on a level surface to allow proper function of the drip management system (drain tube), which you can connect to a waste reservoir to divert solvent leaks from the flow cell.

2.2.2 Power Requirements

The 2487 Detector requires:

- Grounded alternating current (ac) power source.
- Minimal power transients and fluctuations.
- Line voltage of 100 to 240 Vac. Power consumption is 145 volt amps (VA).

The Waters 2487 Detector operates in a nominal voltage range of 100 to 240 Vac, and requires a 3.15 A fuse.



Caution: To avoid electrical shock, power down the 2487 Detector and unplug the power cord before you replace the fuse.



Caution: To reduce the risk of fire hazard, always replace the fuse with the same type and rating.

2.3 Unpacking and Inspecting

The 2487 Detector is packed and shipped in one carton that contains the following items:

- Waters 2487 Dual λ Absorbance Detector
- Startup kit, which includes the Waters 2487 Dual λ Absorbance Detector Operator's Guide
- · Certificate of structural validation
- Power cord
- Release notes

2.3.1 Unpacking

To unpack the 2487 Detector:

- 1. Unpack the contents of the shipping carton. As you unpack the carton, check the contents to make sure you have received all items.
- 2. Check the contents of the startup kit.
- 3. Save the shipping carton for future transport or shipment.

2.3.2 Inspecting

If you see any damage or discrepancy when you inspect the contents of the carton, immediately contact the shipping agent and Waters Technical Service at 1-800-252-4752, *U.S. and Canadian customers only.* Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

Note: Make sure the instrument serial number, found on the detector nameplate on the rear panel or inside the left front panel of the 2487 Detector, corresponds to the number on the instrument validation certificate.

For more information about the instrument warranty, see <u>Appendix C, Warranty</u><u>Information</u>.

2.4 Making Fluidic Connections

Before initial startup of the Waters 2487 Dual λ Absorbance Detector, you must complete:

- 1. The fluidic connections described in this section.
- 2. The electrical connections described in <u>Section 2.5, Making Electrical Power</u> <u>Connections</u>.



Caution: Observe safe laboratory practices when handling solvents. Refer to the Material Safety Data Sheets for the solvents in use.

You need to make the following fluidic connections on your detector unit:

- Column connections
- Drip management system connections

Before completing the column connections, Waters recommends you perform the verification procedures described in <u>Section 3.3.2</u>, <u>Verifying the Detector</u>.

Waters recommends that you install a waste reservoir connected to the drain tube, located next to the rubber foot on the bottom left front of the detector.



Attention: The 2487 Detector, as shipped, has a standard analytical flow cell pressure-rated at 1000 psi. To prevent damage, do not connect any tubing or device that might cause back pressure to exceed the pressure rating of the tubing or flow cell.

Connecting Columns

The fluidic connections to the 2487 Detector are located in the right front of the flow cell assembly (Figure 2-3). To make the inlet and outlet tubing connections:

- 1. Attach the PEEK[™] compression fitting and ferrule (supplied in the 2487 Startup Kit).
- 2. Connect the inlet tubing to the column outlet. Ensure the tubing is seated firmly, then tighten the compression screw.
- 3. Connect the Teflon[®] tubing to the flow cell outlet tubing and route to a waste container.

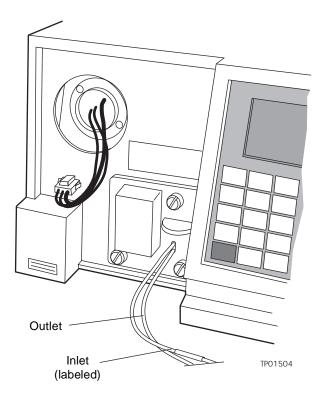


Figure 2-3 2487 Detector Fluidic Connections

Assembling the Fittings

To assemble each fitting:

- 1. Slide the compression screw over the tubing end, followed by the ferrule (Figure 2-4).
- 2. Mount the ferrule with its taper end facing the end of the tubing (Figure 2-4).

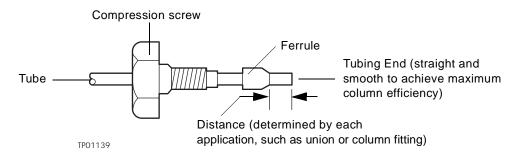


Figure 2-4 Ferrule and Compression Screw Assembly

Making Connections

To make connections at the column outlet and detector inlet, and at the detector outlet:

- 1. Seat each tubing end in the appropriate fitting.
- 2. Seat each ferrule by tightening the compression screw 1/2-turn past finger-tight.

Note: To ensure accurate verification, be sure to power up the 2487 Detector before pumping any mobile phase or solvent through the flow cell.

Note: To prevent reabsorption of dissolved oxygen, for systems using vacuum degassers we recommend that you run the solvent degasser continuously when operating the 2487 Detector at wavelengths less than 230 nm.

2.5 Making Electrical Power Connections

To connect the 2487 Detector to the ac power supply:

- 1. Plug the receptacle end of the power cord into the ac input connector on the rear panel of the detector (Figure 2-5).
- 2. Plug the other end of the power cord into a properly grounded ac power source.

2487 Detector Rear Panel

The 2487 Detector connects to other Waters components through rear panel electrical connections (see Figure 2-5).

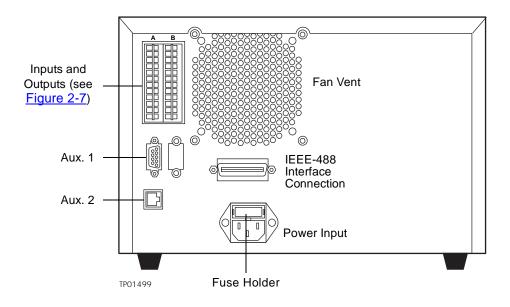


Figure 2-5 2487 Detector Rear Panel Electrical Connections

Rear panel connections enable the following signals:

• Analog outputs – There are two pairs of attenuated analog channel outputs with each pair supporting 2 V and 10 mV outputs to external devices or data systems. They are labeled ChA and ChB. For input/output voltage current specifications, see <u>Table A-1</u>.

Both the 2 V and 10 mV outputs for Channel A and Channel B are scaled based on the AUFS (Absorbance Units Full Scale) setting for each channel. The 2487 Detector does not provide a traditional "unattenuated" dedicated 1 V/AU output because of its enhanced working range above 2 AU.

The 2487 Detector analog output range specifications are as follows:

2 V Output	10 mV Output
– 0.1 V to 2.1 V	– 0.5 mV to 10.5 mV

The 2487 Detector allows the AUFS to be set individually for the output on each channel. Volts per AU are calculated as follows for the 2 V output:

Volts out = Absorbance * 2V/AUFS

For example, an AUFS setting of 2.0000 provides a traditional 1 V/AU output. An AUFS setting of 4.0000 provides a 0.5 V/AU output which supports chromatography above 2 AU.

- Switched outputs There are two switch contact closures you can program to turn on, off, toggle, pulse once for a defined duration, or pulse repetitively for a specified period of time.
- Event inputs There are four general-purpose TTL contact closures on the 2487 Detector A (Inputs) terminal that support the following functions:
 - · Remote or inject start
 - Lamp on/off
 - · Chart mark input
 - Auto zero
- IEEE-488 interface bus The IEEE-488 bus connection, on the rear panel of the detector (see Figure 2-5), allows remote control and direct data acquisition from Waters Millennium®³² and MassLynx[™] workstations, as well as remote control using the Waters Alliance[®] and PowerLine[™] compatible solvent delivery systems.

2.6 Making Signal Connections

The rear panel of the 2487 Detector (see <u>Figure 2-5</u>) provides two analog connectors and an IEEE-488 communications port for operating the detector with external devices.

The signal connections you need to make to your 2487 Detector depend on the:

- Operating mode you select for the 2487 Detector (stand-alone or remote control)
- Types of instruments that make up your HPLC system

This section describes the input/output (I/O) and digital signal connections that you can make from the two rear panel connectors and the IEEE-488 bus connector.

<u>Figure 2-6</u> shows an overview of the steps involved in making signal connections to the 2487 Detector.

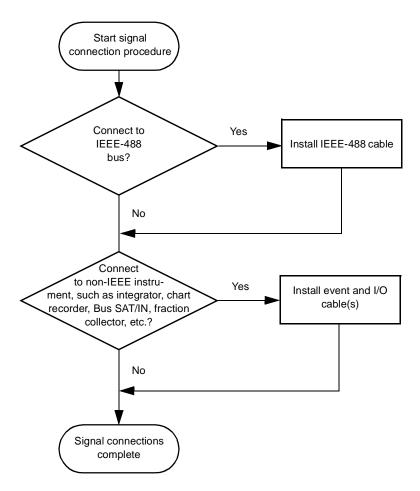


Figure 2-6 Overview of Making Signal Connections

2.6.1 Making I/O Signal Connections

The rear panel includes two removable connectors that hold the pins for the I/O signals, as shown in <u>Figure 2-7</u>. These connectors are keyed so that you can insert them only one way.

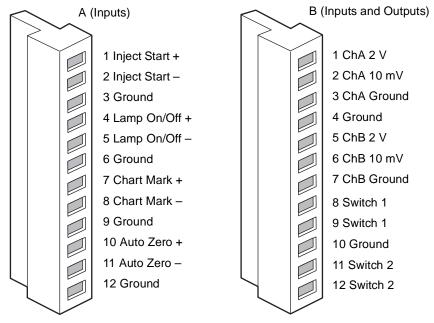


Figure 2-7 I/O Signal Inputs and Outputs

I/O Signals

<u>Table 2-2</u> describes each of the signals available on the I/O connectors. Refer to <u>Appendix A, 2487 Detector Specifications</u>, for details on the electrical specifications for the signals.

Table 2-2 I/O Signals for the 2487 Detector

Signal	Description
Inject Start ¹	TTL contact closure. Configurable input to initiate sequencing of time-programmed events. Defines the start of a run (typically an injection) and resets and starts the run-time clock to 0.00 minutes. Initial conditions apply immediately.
Lamp On/Off ^a	Configurable input to allow an external device to turn the deuterium lamp off and on.
Chart Mark ^a	Configurable input to add a chart mark (at 10% of full scale) to either or both analog output channels (ChA and ChB).

Table 2-2 I/O Signals for the 2487 Detector (Continued)

Signal	Description
Auto Zero ^a	Configurable input to auto zero either or both channels (ChA and ChB).
ChA 2 V ²	2 V full-scale analog output signal of Channel A (scaled to the current AUFS setting).
ChA 10 mV ^b	10 mV full-scale analog output signal of Channel A (scaled to the current AUFS setting).
ChB 2 V ^b	2 V full-scale analog output signal of Channel B (scaled to the current AUFS setting).
ChB 10 mV ^b	10 mV full-scale analog output signal of Channel B (scaled to the current AUFS setting).
Switch 1 (2)	Used to connect to a fraction collector. Can be controlled by threshold and timed events.
Switch 2 (2)	

¹ Inject start, chart mark, auto zero, and lamp inputs are configurable from the 2487 Detector second Configuration screen by setting the appropriate parameter to High. See <u>"Configuring Event Inputs</u> (Contact Closures)" in Section 3.2.6, Operating Other Detector Functions, for more detail. ² See the discussion of attenuation in the 2487 Detector analog outputs in <u>Section 2.5, Making Electrical Power Connections</u>.

2.6.2 Connecting the 2487 Detector to a 2690 Separations Module in a Stand-Alone Configuration

You can connect the 2487 Detector to the Waters 2690 Separations Module, when it is not under the control of the Millennium³² software, to perform the following:

- Auto zero on inject
- Chart mark on inject
- Method start
- Turn lamp on and off

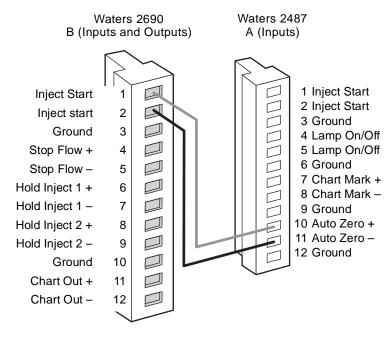
Generating Auto Zero on Inject

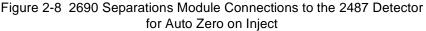
To generate the auto zero function on the 2487 Detector at the start of an injection, make the connections summarized in <u>Table 2-3</u> and illustrated in <u>Figure 2-8</u>.

Table 2-32487 Detector Connections to a Waters 2690 Separation Module to GenerateAuto Zero on Inject

2690 Separations Module (B Inputs and Outputs)	2487 Detector (A Inputs)
Pin 1 Inject Start	Pin 10 Auto Zero +
Pin 2 Inject Start	Pin 11 Auto Zero –

Before you can generate an auto zero from the 2690 Separations Module, you need to configure the auto zero signal at the 2487 Detector front panel. The default auto zero signal is Low. See the discussion <u>"Configuring Event Inputs (Contact Closures)</u>" in <u>Section 3.2.6, Operating Other Detector Functions</u>, for more information.





Generating Chart Mark on Inject

To generate the chart mark function on the 2487 Detector at the start of an injection, make the connections summarized in <u>Table 2-4</u> and illustrated in <u>Figure 2-9</u>.

Table 2-42487 Detector Connections to a Waters 2690 Separation Module to GenerateChart Mark on Inject

2690 Separations Module (B Inputs and Outputs)	2487 Detector (A Inputs)
Pin 1 Inject Start	Pin 7 Chart Mark +
Pin 2 Inject Start	Pin 8 Chart Mark –

Before you can generate a chart mark from the 2690 Separations Module, you need to configure the chart mark signal at the 2487 Detector front panel. The default chart mark signal is Low. See the discussion <u>"Configuring Event Inputs (Contact Closures)"</u> in <u>Section 3.2.6, Operating Other Detector Functions</u>, for more information.

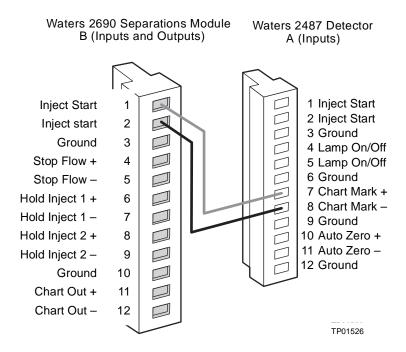


Figure 2-9 2690 Separations Module Connections to the 2487 Detector for Chart Mark on Inject

Starting a Method

To start a method on the 2487 Detector at the start of an injection from the 2690 Separations Module, make the connections summarized in <u>Table 2-5</u> and illustrated in <u>Figure 2-10</u>.

Table 2-5 2487 Detector Connections to a Waters 2690 Separation Module to Start a Method

2690 Separations Module (B Inputs and Outputs)	2487 Detector (A Inputs)
Pin 1 Inject Start	Pin 1 Inject Start +
Pin 2 Inject Start	Pin 2 Inject Start –

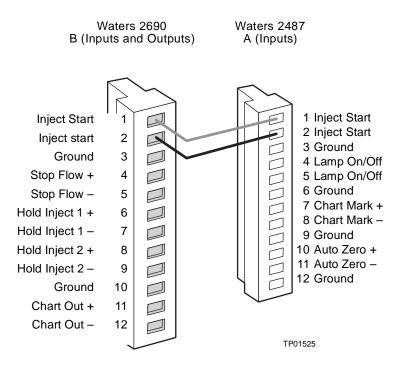


Figure 2-10 2690 Separations Module Connections to the 2487 Detector for Starting a Method

Turning the 2487 Detector Lamp On or Off

Before you can turn the 2487 Detector lamp on or off from the 2690 Separations Module, you need to configure the lamp on/off signal at the front panel. You must change the default lamp configuration parameter from Ignore to High or Low. See the discussion "Configuring Event Inputs (Contact Closures)" in Section 3.2.6, Operating Other Detector Functions, for more information. After configuring the 2487 Detector lamp on/off signal, you can turn the lamp on or off from the 2690 Separations Module by making the connections in <u>Table 2-6</u> and shown in <u>Figure 2-11</u>.

2690 Separations Module (A Outputs)	2487 Detector (A Inputs)
Pin 1 Switch 1	Pin 4 Lamp On/Off +
Pin 2 Switch 1	Pin 5 Lamp On/Off –

Table 2-6 2487 Detector Connections to a Waters 2690 Separation Module to Turn the Detector Lamp On or Off

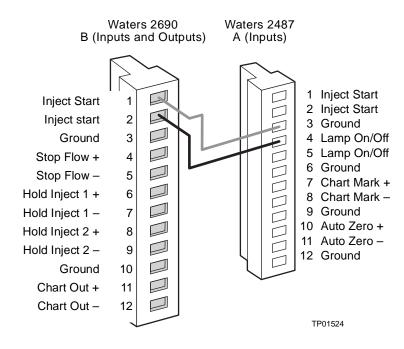


Figure 2-11 2690 Separations Module Connections to the 2487 Detector for Turning the Lamp On or Off

2.6.3 Making IEEE-488 Connections in Breeze and Millennium³²

The rear panel also includes one IEEE-488 interface connector for digital signal communications. Use the IEEE-488 interface connector to connect the 2487 Detector to IEEE-488 devices, such as:

- busLAC/E[™] card in the Millennium³² workstation or a LAC/E³² module
- Breeze system
- PowerLine controller
- Alliance systems (2690, 2690D, or 2790 Separations Module)
- MassLynx version 3.5x or higher

The IEEE-488 connector mates with a standard IEEE-488 cable.

Attention: To avoid possible damage to components, power off all instruments on the IEEE-488 control bus before you connect an IEEE-488 interface cable to an instrument.

Note: When the 2487 Detector is operating in dual wavelength mode and connected to Millennium³² software versions lower than 3.0, PowerLine, or a Waters 2690 Alliance Separations Module version 1.15 or lower using the IEEE-488 communications bus, you must enable the **Emulate dual 486** configuration option.

Making IEEE-488 Connections with Waters Data Systems

When controlling the 2487 Detector from a Waters data system or controller (Millennium³² or MassLynx workstation, PowerLine, or a 2690, 2690D, or 2790 Alliance HT Separations Module), you can use the IEEE-488 interface to send and receive information from the data system.

When operating with these systems, the 2487 Detector emulates two separate 486 Detectors on the IEEE-488 communications bus, when the Detector is in dual wavelength mode. When connecting via the IEEE-488 to these Waters data systems, you should be aware that:

- The 2487 Detector reports its 486 Detector version number as 5.0 in Millennium³² systems below Release 3.05.01.
- When in dual wavelength mode, you must select a data rate of 1 point per second in the data system method editor. In the Millennium³² software, the data rate parameters defaults to 1 point per second.
- The default AUFS setting is 1.000 AUFS. This results in a 2-V/AU scaled output. If a 1-V/AU scaled output is desired, set the AUFS to 2.000.

- Millennium³² software releases below 3.05.01 support wavelengths from 190 to 600 nm, not the full range of 100 to 700 nm of the 2487 Detector.
- Millennium³² software releases below 3.05.01 support a maximum AUFS setting of 2.000, not the full 4.000 AUFS of the 2487 Detector.
- Millennium³² software releases below 3.05.01 support a maximum time constant of 5.0 seconds, regardless of filter type. The maximum range of the 2487 Detector time constant setting depends on the wavelength mode and filter type selected (see <u>Table 3-3</u>).
- Millennium³² version 3.05.01 and higher allows the 2487 Detector to operate in both single and dual wavelength modes with a wavelength range of from 1 to 700 nm, and up to 4.0 AUFS.

To connect IEEE-488 cables from the 2487 Detector to a Waters data system:

- 1. Connect the single receptacle end of the IEEE-488 cable (supplied with the Waters data system) to your data system:
 - Millennium or Millennium³² Chromatography Manager Attach the cable to the busLAC/E (laboratory acquisition and control environment) or the LAC/E³² card (Figure 2-12).
 - **Breeze system** Attach the cable to the busLAC/E (laboratory acquisition and control environment).
 - Alliance (Waters 2690, 2690D, or 2790 Separations Module and 2487 Detector) systems – Attach the cable directly from the 2690, 2690D, or 2790 Separations Module to the 2487 Detector (Figure 2-13). For connections from the 2690 Separations Module to the 2487 Detector in a stand-alone configuration, see Section 2.6.2, Connecting the 2487 Detector to a 2690 Separations Module in a Stand-Alone Configuration. For connections from the 2690D or the 2790 Separations Module to the 2487 Detector, see the 2690D or 2790 operator's guide.
- 2. Connect the other end of the cable to the IEEE-488 connector on the 2487 Detector rear panel.
- 3. If you are using the 2487 Detector as part of a multicomponent configuration (see <u>Figure 2-12</u> or <u>Figure 2-13</u>), connect a second IEEE-488 cable to the stackable connector on the detector. Connect the other end of the cable to the IEEE-488 port on the next component.
- 4. Repeat steps 2 and 3 for each additional component.



Attention: The maximum total cable length between IEEE-488 devices in a system is 65 feet (20 meters). The maximum recommended cable length between two IEEE-488 devices is 13 feet (4 meters). Longer total cable lengths can cause intermittent IEEE-488 communication failures.

- 5. Ensure all IEEE-488 cable screws are fastened finger-tight.
- 6. Set a unique IEEE-488 address from 2 to 29 for each device connected to the IEEE-488 bus when operating the 2487 Detector in single wavelength mode. The default IEEE-488 address for the 2487 Detector is 4. Refer to the "Dual Wavelength Mode" discussion in this section for information on setting IEEE-488 addresses while in dual wavelength mode.

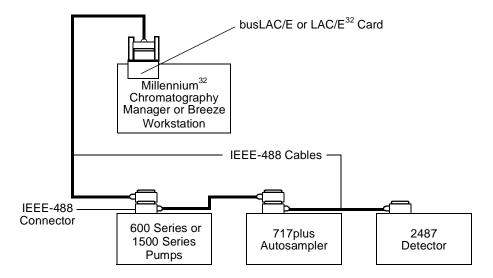


Figure 2-12 IEEE-488 Connections to the 2487 Detector in a Waters Millennium³² System

Note: When connecting the 2487 Detector to a data system, all detector parameters not configurable by the data system in use defer to local control.

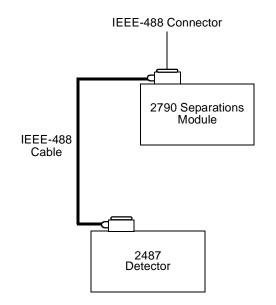


Figure 2-13 IEEE-488 Connections to the 2487 Detector in an Alliance System

Making IEEE-488 Connections with PowerLine Controllers

If you are controlling the 2487 Detector from a PowerLine controller, use the IEEE-488 interface to communicate with the detector (the PowerLine controller manages the IEEE-488 interface functions).

To set up the PowerLine controller, refer to the *Waters 600E Multisolvent Delivery System User's Guide*, Section 4.1, Setting Up the 600 as a PowerLine Controller, or to the specific PowerLine component operator's guide.

To connect IEEE-488 cables:

- 1. Connect the single-receptacle end of the IEEE-488 cable (supplied with the PowerLine system) to the IEEE-488 connector on the controller rear panel (refer to the specific controller operator's guide).
- 2. Connect the other end of the cable (stackable connector for daisy-chaining additional instruments) to the IEEE-488 connector on the 2487 Detector.
- If you are using a multicomponent configuration (shown in <u>Figure 2-14</u>), connect a second IEEE-488 cable to the stackable connector on the PowerLine instrument. Connect the other end of the cable to the IEEE-488 port on the next PowerLine component. <u>Figure 2-14</u> illustrates IEEE-488 cable connections in a PowerLine system.

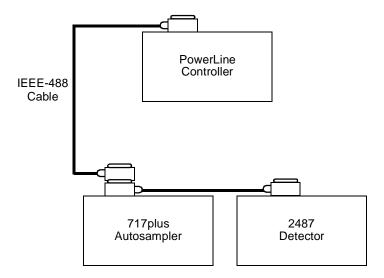


Figure 2-14 PowerLine Controller IEEE-488 Connections

4. Repeat steps 2 and 3 for each additional PowerLine component.



Attention: The maximum total cable length between IEEE-488 devices is 65 feet (20 meters). The maximum recommended cable length between two IEEE-488 devices is 13 feet (4 meters). Longer total cable lengths can cause intermittent IEEE-488 communication failures.

- 5. Ensure all IEEE-488 cable screws are fastened finger-tight.
- 6. Set a unique IEEE-488 address from 2 to 29 for each device connected to the IEEE-488 bus.

Setting IEEE-488 Addresses

When operating the detector in single wavelength mode, you must set a unique IEEE-488 address from 2 to 29 for the 2487 Detector to be recognized on the IEEE-488 interface, busLAC/E card, or LAC/E³² module. Set the IEEE-488 address from the front panel of the 2487 Detector. The default IEEE-488 address for the 2487 Detector is 4.

Note: When the 2487 Detector is operating in dual wavelength mode and connected to Millennium³² software versions lower than 3.0, PowerLine, or a Waters 2690 Alliance Separations Module version 1.15 or lower using the IEEE-488 communications bus, you must enable the **Emulate dual 486** configuration option.

The IEEE-488 address you assign to the detector must be an even number from 2 to 28 (the detector assigns the next consecutive address to the "second" 486 Detector). The default IEEE-488 address for the 2487 Detector is 4.

Single Wavelength Mode

To set or change an IEEE-488 address:

- 1. Press **CONFIGURE** (Shift DIAG) on the 2487 Detector keypad. The first configuration screen containing the current IEEE-488 address appears.
- 2. Key in the desired IEEE-488 address.
- 3. Press Enter. The IEEE-488 address is entered immediately (power reset is not required).
- 4. Press **Next** to move to the next configuration screen or press **HOME** to return to the Absorbance screen.

Dual Wavelength Mode

When the 2487 Detector is operating in dual wavelength mode under the control of PowerLine or the 2690 Separations Module, it appears on the controller configuration screen as two separate 486 Detectors if the **Emulate dual 486** configuration option is enabled (see the discussion <u>"Configuring the 2487 Detector"</u> in <u>Section 3.2.6, Operating Other Detector Functions</u>).

When operating in dual wavelength mode, the 2487 Detector uses the defined IEEE-488 address and the next consecutive address. The IEEE-488 address defined for the "first" 486 Detector must be an even number from 2 to 28 for the detector to operate correctly in dual wavelength mode (the detector assigns the next consecutive address to the "second" 486 Detector). The default IEEE-488 address for the 2487 Detector is 4.

Follow the steps in "Single Wavelength Mode" to set the IEEE-488 address. When you press **Enter** to enter the first IEEE address, the detector displays the next consecutive address automatically if **Emulate dual 486** is enabled.



Attention: To prevent acquisition of invalid data when operating the 2487 Detector in dual wavelength mode under the control of the Millennium³² Chromatography Manager or a PowerLine system, you must select a data sampling rate of 1 point per second.

2.7 Connecting to Other Devices

You can connect the 2487 Detector to a wide range of HPLC system devices. This section describes how to connect the 2487 Detector to the following devices:

- Millennium³² using the bus SAT/IN[™] Module
- Waters 745/745B/746 Data Module
- · Chart recorder
- Waters 600 Series Pump
- Waters 717plus Autosampler
- · Waters fraction collector

Required Materials

When connecting cables to the terminals on the rear panel of the 2487 Detector, you need the following tools:

- Small flat-blade screwdriver
- Electrical insulation stripping tool

Connecting the Cables

To connect cables from other HPLC system devices to the A and B terminals on the rear panel of the 2487:

- 1. Remove terminal A or B (see Figure 2-7).
- 2. Unscrew the connecting pin terminal.
- 3. Using the stripping tool, strip the wire down about 1/8-inch from the end.
- 4. Insert the stripped wire into the appropriate connector.
- 5. Tighten the screw until the wire is held securely in place.
- 6. Reinsert the terminal.
- 7. Press firmly to ensure that it is inserted fully.

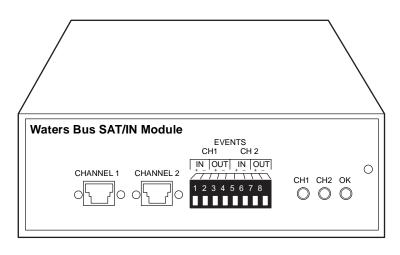
2.7.1 Connecting the 2487 Detector to the Millennium³² System Using the busSAT/IN Module

To acquire data and control the 2487 Detector with the Millennium³² software using the busSAT/IN Module instead of the IEEE-488 bus (see <u>Section 2.6.3</u>, <u>Making IEEE-488</u> <u>Connections in Breeze and Millennium³²</u>) requires connections between the following hardware:

- Laboratory acquisition and control environment (LAC/E) module (LAC/E³² Acquisition Server or busLAC/E card)
- Satellite interface (SAT/IN) module

busSAT/IN Module

The Waters busSAT/IN module shown in <u>Figure 2-15</u> translates analog signals from devices such as the 2487 Detector into digital form. It then transmits these digital signals to the busLAC/E or LAC/E³² card installed in the Millennium³² workstation.



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To connect the 2487 Detector to the Millennium³² workstation:

1. Connect the busSAT/IN Module to the busLAC/E or LAC/E³² card in the Millennium³² computer, according to the instructions in the *Waters Bus SAT/IN Module Installation Guide.*

 Connect the Waters 2487 Detector to the busSAT/IN Module (see the following discussion, <u>"Connecting the 2487 Detector to the busSAT/IN Module"</u>).



Attention: To ensure proper startup of the busSAT/IN Module, do not turn power on to the module until you perform all procedures described in the Waters Bus SAT/IN Module Installation Guide. Improper startup can damage the unit and void the warranty.



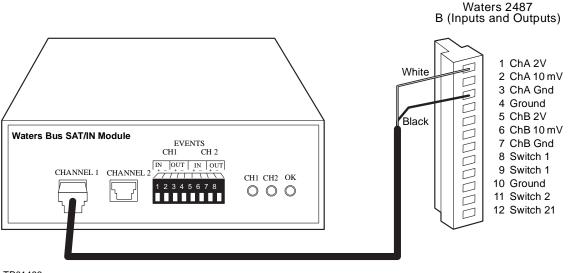
Attention: The busSAT/IN Module does not have a power switch. To prevent damage to the module, always disconnect the power cord at either the wall outlet or the power supply before attaching or removing the power connection to the busSAT/IN Module.

Connecting the 2487 Detector to the busSAT/IN Module

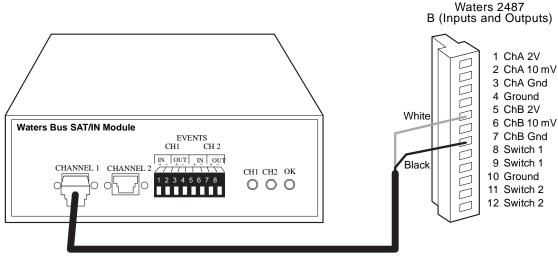
The busSAT/IN Module connects to the 2487 Detector through the B (Inputs and Outputs) terminal on the rear panel of the detector, as shown in <u>Figure 2-16</u>.

To connect the 2487 Detector to the busSAT/IN Module:

- 1. Using the electrical insulation stripping tool, strip about 1/8 inch off one end of the busSAT/IN 9-pin connector, exposing the white and black wires.
- 2. Connect the other end of the cable to either the Channel 1 or Channel 2 connectors on the front panel of the busSAT/IN Module.
- 3. For Channel A (see Figure 2-16):
 - a. Connect the white wire to pin 1 on B (ChA 2 V).
 - b. Connect the black wire to pin 3 on B (ChA Ground).
- 4. For Channel B (see Figure 2-16):
 - a. Connect the white wire to pin 5 on B (ChB 2 V).
 - b. Connect the black wire to pin 7 on B (ChB Ground).
- 5. Configure the serial port for the busSAT/IN Module as described in the *Millennium*³² System Installation/Configuration Guide.



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Figure 2-16 busSAT/IN Module Channel 1 Connection to 2487 Detector Channel A

Table 2-7 summarizes the 2487 Detector connections to the busSAT/IN Module.

Table 2-7 2487 Detector Connections to a busSAT/IN Module

2487 Detector (B Inputs and Outputs)	busSAT/IN Connector	
Pin 1 ChA 2 V (white)	Channel 1 as 2	
Pin 3 ChA Ground (black)	Channel 1 or 2	
Pin 5 ChB 2 V (white)	Channel 1 or 2	
Pin 7 ChB Ground (black)	Channel 1 or 2	

2.7.2 Connecting the 2487 Detector to a 745/745B/746 Data Module

You can connect the 2487 Detector to a Waters 745/745B/746 Data Module using the analog output connector on the rear panel of the detector. The analog connector provides both 2 V and 10 mV outputs which are scaled to the AUFS sensitivity setting and the voltage offset setting.



Attention: To prevent oversaturation of the signal from the 2487 Detector to the 745/745B/746 integrator, do not exceed the input voltage rating of the integrator.

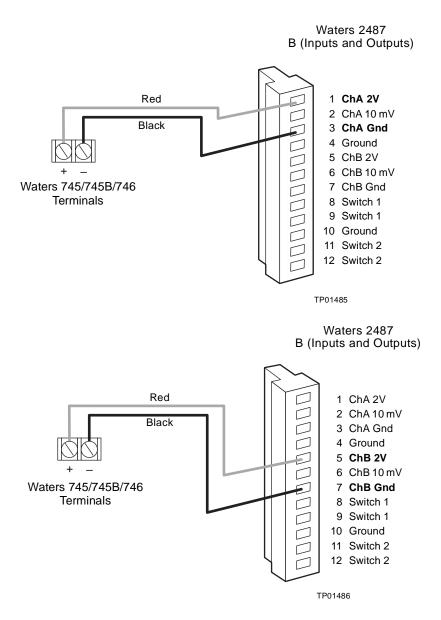
To send the analog output signal from the 2487 Detector to the Waters 745/745B/746 Data Module, use the cable provided in the 2487 Detector Startup Kit to make the connections summarized in <u>Table 2-8</u> and illustrated in <u>Figure 2-17</u>.

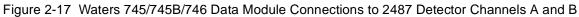
Table 2-8 2487 Detector Connections to a Waters 745/745B/746 Data Module

2487 Detector (B Inputs and Outputs)	745/745B/746 Terminal
Pin 1 ChA 2 V (red)	+
Pin 3 ChA Ground (black)	-
Pin 5 ChB 2 V (red)	+
Pin 7 ChB Ground (black)	-



Attention: To minimize the chance of creating a ground loop that can adversely affect measurement, connect the shield of the cable to the chassis ground at one end only.





2.7.3 Connecting the 2487 Detector to a Chart Recorder

Recorder Signal

The A and B terminals on the rear panel of the 2487 Detector provide 2 V and 10 mV analog output signals that you can use to connect the 2487 Detector to a chart recorder.

To send 2 V or 10 mV signals from the 2487 Detector to a chart recorder, use the cable provided in the 2487 Startup Kit to make the connections summarized <u>Table 2-9</u> and illustrated in <u>Figure 2-18</u>.

2487 Detector (B Inputs and Outputs)	Chart Recorder Terminal
Pin 1 ChA 2 V or Pin 2 ChA 10 mV	+
Pin 3 ChA Ground	_
Pin 5 ChB 2 V or Pin 6 ChB 10 mV	+
Pin 7 ChB Ground	-

Table 2-9 2487 Detector Connections to a Chart Recorder



Attention: To minimize the chance of creating a ground loop that can adversely affect measurement, connect the shield of the cable to the chassis ground at one end only. For connection to other data systems, a cable (P/N 44000172) can help remove the incidence of ground loops.

Note: The 2487 Detector is optimized for use with the 2 V analog output. Use the 10 mV analog output only if necessary.

Connect the 2487 Detector to a chart recorder using the 2 V analog connections as shown in Figure 2-18.

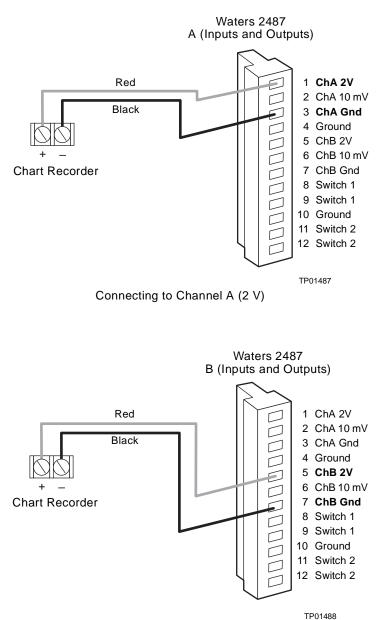
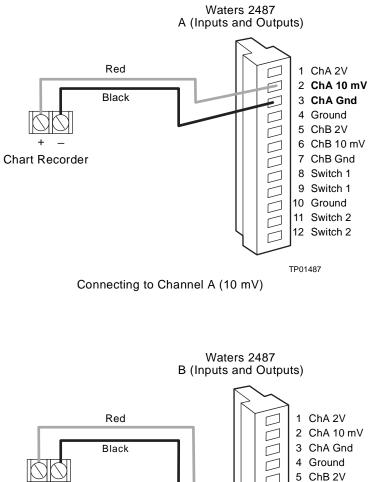




Figure 2-18 Chart Recorder 2 V Output Connections on 2487 Detector Channels A and B



Connect the 2487 Detector to a chart recorder using the 10 mV analog connections as shown in <u>Figure 2-19</u>.

Red Black chA Gnd chA Gnd chA Gnd chA Gnd chB 2V chA B 0 mV chart Recorder chart Recorder chart 1 ChA 2V chA Gnd chB 10 mV chart Recorder chart 1 ChA 2V chA Gnd chB 10 mV chart 1 ChA 2V chA Gnd chB 2V chB Gnd s Switch 1 chB Switch 2 chB 2V chB Gnd s Switch 1 chB Switch 2 chB 2V chB Gnd s Switch 1 chB Switch 2 chB 2V chB 2V chB Switch 2 chB 2V chB 2V chB Switch 2 chB 2V chB

Connecting to Channel B (10 mV)

Figure 2-19 Chart Recorder 10 mV Output Connections on 2487 Detector Channels A and B

Chart Marks

You can also generate a chart mark from the front panel of the 2487 Detector. A chart mark signal is generated whenever one of the following occurs:

- You press the Chart Mark key on the detector keypad.
- A chart mark signal is generated at the chart mark inputs on the analog connector.
- You program a timed event to generate a chart mark.

2.7.4 Connecting the 2487 Detector to the Waters 600 Series Pump

To connect the 2487 Detector to the Waters 600 Series Pump, locate the 2487 Detector in a position that satisfies the site requirements in <u>Section 2.2, Site Selection and Power</u> <u>Requirements</u>.

Fluid Connections

Make the fluid connections described in Section 2.4, Making Fluidic Connections.

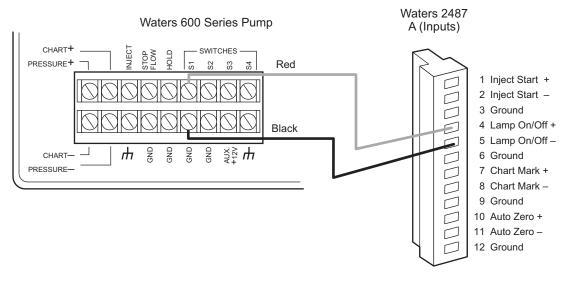
Lamp On/Off Connections

In addition to making the connections described below, you must configure the Lamp On/Off signal at the 2487 Detector front panel. You must change the default from Ignore to High or Low. See the discussion <u>"Configuring Event Inputs (Contact Closures)</u>" in <u>Section 3.2.6, Operating Other Detector Functions</u>, for more information.

Using signal cable, make the connections between the Waters 600 Series Pump controller and the 2487 Detector that are summarized in <u>Table 2-10</u> and shown in <u>Figure 2-20</u>.

Table 2-10 Waters 600 Series Pump and 2487 Detector Lamp On/Off Connections

Waters 2487 Detector A (Inputs)	Waters 600 Series Pump Terminal
Pin 4 Lamp On/Off +	S1, S2, or S4
Pin 5 Lamp On/Off –	GND



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Auto Zero Connections

To make auto zero connections between the 2487 Detector and the 600 Series Pump:

- Using a signal cable, make the connections between the 2487 Detector and the Waters 600 Series Pump that are summarized in <u>Table 2-11</u> and illustrated in <u>Figure 2-21</u>.
- Program the 600 Series Pump to provide a pulse output on the applicable switch (S1, S2, or S4) at the beginning of each run. Refer to the *Waters 600E Multisolvent Delivery System User's Guide*, Section 5.1.2, for more details.

Table 2-11 Waters 600 Series Pump and 2487 Detector Auto Zero Connections

Waters 2487 Detector A (Inputs)	Waters 600 Series Pump Terminal
Pin 10 Auto Zero +	S1, S2, or S4
Pin 11 Auto Zero –	GND (one of four)

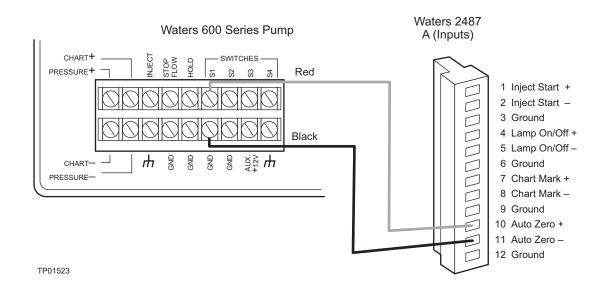




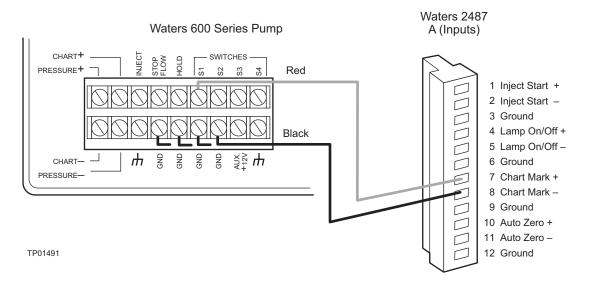
Chart Mark Connections

To make chart mark connections between the 2487 Detector and the 600 Series Pump:

- Using a signal cable, make the connections between the 2487 Detector and the Waters 600 Series Pump that are summarized in <u>Table 2-12</u> and illustrated in <u>Figure 2-21</u>.
- 2. Program the 600 Series Pump to provide a pulse output on the selected switch at the beginning of each run. Refer to the *Waters 600E Multisolvent Delivery System User's Guide*, Section 5.1.2, for more details.

Table 2-12 Waters 600 Series Pump and 2487 Detector Chart Mark Connections

Waters 2487 Detector A (Inputs)	Waters 600 Series Pump Terminal
Pin 7 Chart Mark +	S1, S2, or S4
Pin 8 Chart Mark –	GND (one of four)





Inject Start Connections

Note: If the 2487 Detector is connected to a Millennium³² data system, using these connections allows the 2487 Detector to initiate the start of data acquisition.

To make inject start connections between the Waters 600 Series Pump and the 2487 Detector to start a method:

- Using a signal cable, make the connections between the 2487 Detector and the Waters 600 Series Pump that are summarized in <u>Table 2-13</u> and illustrated in <u>Figure 2-23</u>.
- 2. Program the 600 Series Pump to provide a pulse output on the selected switch at the beginning of each run. Refer to the *Waters 600E Multisolvent Delivery System User's Guide,* Section 5.1.2, for more details.

Table 2-13 Waters 600 Series Pump and 2487 Detector Inject Start Connections

Waters 2487 Detector A (Inputs)	Waters 600 Series Pump Terminal
Pin 1 Inject Start +	S1, S2, or S4 ¹
Pin 2 Inject Start –	GND (one of four)

¹ You may also connect the Waters 600 Series Pump Inject pin to the Pin 1 Inject Start + on the 2487 Detector, and the Inject Ground pin to the Inject Start – on the 2487 Detector.

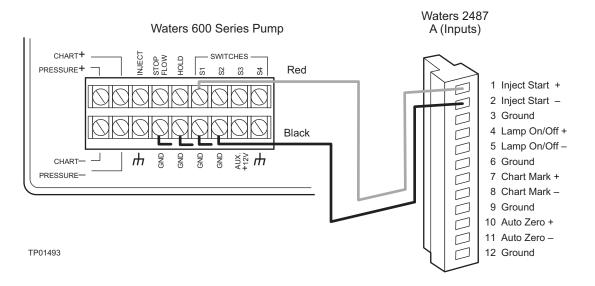


Figure 2-23 Waters 600 Series Pump Inject Start Connections

2.7.5 Connecting the 2487 Detector to the Waters 717plus Autosampler

The Waters 717plus Autosampler signals the start of an injection through a contact closure signal on its Inject Start terminals. You can use this contact closure signal to command the 2487 Detector to perform an auto zero at the start of an injection.

Auto Zero Connections

To auto zero the 2487 Detector at the start of an injection, make the connections described in <u>Table 2-14</u> and illustrated in <u>Figure 2-24</u>.

2487 Detector (A Inputs)	717plus Autosampler Terminal
Pin 10 Auto Zero +	Inject Start + (any one of three paired with –)
Pin 11 Auto Zero –	Inject Start – (any one of three paired with +)

Table 2-14 Waters 717 plus Autosampler and 2487 Detector Auto Zero Connections

<u>Figure 2-24</u> illustrates the connections between the 2487 Detector and the Waters 717plus Autosampler. Use any available pair of Inject Start terminals on the 717plus Autosampler.

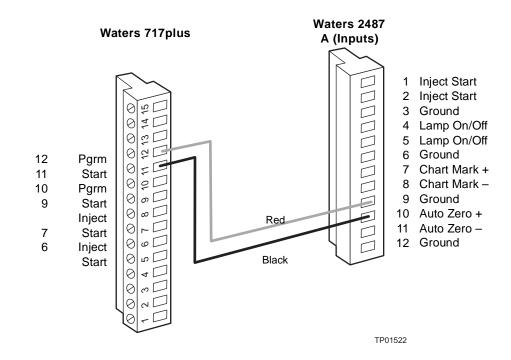


Figure 2-24 717 plus Auto Zero Connections to the 2487 Detector

Inject Start Connections

You can also use the Inject Start connections on the 717plus Autosampler to connect to the Inject Start signals on the 2487 Detector to program the start of the active method.

To program an inject start, make the connections described in <u>Table 2-15</u> and illustrated in <u>Figure 2-25</u>.

Table 2-15 Waters 717 plus Autosampler and 2487 Detector Inject Start Connections

2487 Detector (A Inputs)	717plus Autosampler Terminal
Pin 1 Inject Start +	Inject Start + (any one of three paired with +)
Pin 2 Inject Start –	Inject Start – (any one of three paired with –)

Figure 2-25 illustrates the connections between the 2487 Detector and the Waters 717plus Autosampler. Use any available pair of Inject Start terminals on the 717plus Autosampler.

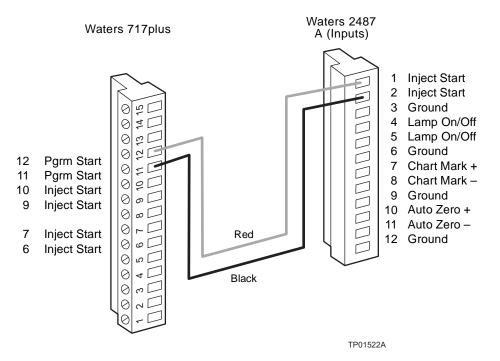


Figure 2-25 Waters 2487 Detector Inject Start Connections

2.7.6 Connecting the 2487 Detector to a Fraction Collector

The 2487 Detector can trigger a fraction collector based on:

- Timed events (see the discussion <u>"Timed Events</u>" in <u>Section 3.3.6</u>, <u>Programming</u> <u>Timed Events</u>, <u>Threshold Events</u>, <u>and Methods</u>)
- Threshold levels (see the discussion <u>"Threshold Events</u>" in <u>Section 3.3.6,</u> <u>Programming Timed Events, Threshold Events, and Methods</u>)

You can connect the fraction collector to one of the 2487 Detector's two programmable switches (SW1 or SW2) and program the timed event, threshold, or ratio at the detector front panel.

You can also connect the fraction collector to the 2487 Detector to trigger a chart mark event input each time a tube is changed at the fraction collector.

See <u>Table 2-16</u> for the correct detector-to-fraction collector and autoinjector-to-fraction collector connections.

Table 2-16	Fraction	Collector to	2487	Detector	Connections
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Waters 2487 Connection	Waters Fraction Collector	
B (Inputs and Outputs) Pin 2 ChA 10 mV	Pin 2 Detector In +	
B (Inputs and Outputs) Pin 3 ChA Ground	Pin 1 Detector In –	
A (Inputs) Pin 7 Chart Mark +	Pin 10 Event Marker +	
A (Inputs) Pin 8 Chart Mark –	Pin 9 Event Marker –	
B (Inputs and Outputs) Pin 8 SW1	Pin 7 External Count In +	
B (Inputs and Outputs) Pin 10 Ground	Pin 8 External Count In –	
Waters 2690 Separations Module/717plus Autoinjector		
Inject Start +	External Start In +	
Inject Start –	External Start In –	

Note: Please refer to the documentation provided with your fraction collector for complete information.

3 Using the 2487 Detector

After you have installed the Waters[®] 2487 Dual λ Absorbance Detector, you are ready to operate the 2487 Detector.

Note: To ensure accurate operation, and before pumping any mobile phase or solvent through the flow cell, be sure to perform the procedures in <u>Section 3.3.2</u>, <u>Verifying the</u> <u>Detector</u>.

Using the Detector as a Stand-Alone Instrument

You can use the Waters 2487 Detector as a stand-alone UV/Vis detector in conjunction with a pump, injector, chart recorder, or integrator. To use the detector in one of these configurations, follow the instructions in this chapter.

You can program the 2487 Detector from the front panel except when it is in remote mode. Refer to <u>Section 3.3, Operating the 2487 Detector</u>, for more information.

Using the Detector as Part of a System

You can also use the 2487 Detector configured with a system controller, such as the Waters Millennium[®]³² Chromatography Manager, Waters Alliance[®] (2690, 2690D, or 2790) Separations Module, or a Waters PowerLine[™] system.

To configure the Waters 2487 Detector with one of these systems, follow the instructions in the appropriate system operator's guide to set the parameters and control the detector. You also need to set the IEEE-488 address (see the discussion <u>"Configuring the 2487</u> <u>Detector"</u> in <u>Section 3.2.6</u>, <u>Operating Other Detector Functions</u>) on the 2487 Detector to configure the detector for remote operation.

3.1 Starting Up the 2487 Detector

3.1.1 Initializing the 2487 Detector

Before you power on the Waters 2487 Detector, be sure the connector from the 2487 Detector rear panel to the power source is properly installed.

To turn power on, press the **On/Off** switch located on the lower-front right corner of the unit.

At startup, the 2487 Detector beeps three times and runs a series of startup diagnostic tests. If all startup diagnostics pass, the startup diagnostics OK messages are displayed (Figure 3-1).

STAR	STARTUP DIAGNOSTICS		
TPU	ОК	ROM OK	
SCI	ОК	RAM OK	
GPIB	ОК	LCD OK	
QSPI	OK	CPU OK	

Figure 3-1 Waters 2487 Detector Startup Diagnostics Display

Following display of the Startup Diagnostics screen, the detector displays the following series of messages in sequence for about five minutes:

- Initializing grating
- · Initializing system
- Lighting lamp
- Warmup time left: n minutes
- Homing optical filter
- · Searching for 656 nm
- Optimizing system performance
- Finding calibration peaks
- · Restoring last setup
- Completing initialization

When initialization is complete, the 2487 Detector displays the Absorbance screen (Figure 3-2). Section 3.2.2, Using the Keypad, and Section 3.2.3, Navigating the User Interface, provide more information on this and subsequent screens.

Note: For normal use, allow the detector to warm up for at least 30 minutes before operating.

3.1.2 Diagnostics Failure

If one or more of the internal startup diagnostics checks fails, the detector:

- Beeps
- Displays an error message
- For serious errors, it displays the word "Error" in brackets (<Error>) in place of the run-time absorbance on the Absorbance screen.

See <u>Section 5.1, Error Messages</u>, for a list of startup diagnostics failures, error messages, and recommended recovery actions. See <u>Section 5.3.2</u>, <u>Hardware Troubleshooting</u>, for hardware-related causes of startup diagnostics failure and corrective actions.

3.2 Using the Operator Interface

3.2.1 Using the Display

The 2487 Detector employs a 128 x 64 bitmap graphic display and a 24-key membrane keypad for the operator interface. After the startup diagnostics have run successfully, the 2487 Detector displays the Absorbance (or HOME) screen (Figure 3-2).

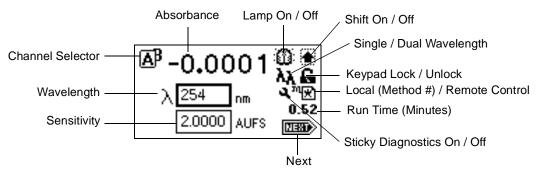


Figure 3-2 2487 Detector Absorbance Screen

You can recall the Absorbance screen at any time by pressing the **HOME** key. At the first use of the detector, the Absorbance screen shows the factory-set defaults. After the first use, the Absorbance screen shows the settings displayed before the 2487 Detector was last powered down. The Absorbance screen continues to change as the run continues.

The detector monitors absorbance of one or two wavelengths in real time, while allowing you to modify all the parameters discussed in <u>Table 3-1</u>. You can use the A/B key to toggle between Absorbance screens for Channels A and B.

Absorbance and Message Icons

The Absorbance screens and message screens in the 2487 Detector program display the icons or fields shown in <u>Figure 3-2</u> and described in <u>Table 3-1</u>. For a list of ranges and defaults for the function icons and fields described in <u>Table 3-1</u>, see <u>Table 3-3</u>.



Attention: Changing the sensitivity (AUFS) setting affects both the 2 V and 10 mV outputs. For example, 1 AU on a 2 V output gives 0.5 AU/V and 2 AU on a 2 V output gives 1 AU/V.

Table 3-1 Absorbance and Message Screen Icons

Icon or Field	Icon / Field Name	Function
Field requiring entry	Sensitivity or AUFS	Selects the chart sensitivity in absorbance units full scale (AUFS) for the selected channel (IEEE signal is not affected).
Field requiring entry	Wavelength	Selects the wavelength monitored on the selected channel. When in single wavelength mode there is no independent control of wavelength on Channel B.
AB SB B	Channel Selector	Displays which channel is selected when you press the A/B key. The selected channel overlaps the other channel. Displays the ON A or ON B icon for the channel on which a timed or threshold event is programmed. When you press the TRACE key, displays only the channel being viewed.
Numerical field	Absorbance	Displays current absorbance for the selected channel.
	Lamp On / Off	On = Lamp icon Off = Lamp icon with an X through it

Icon or Field	Icon / Field Name	Function
	Shift On / Off	Blank = Shift off s = Shift on
λ λλ	Single / Dual Wavelength	λ = Detector operating in single wavelength mode $\lambda\lambda$ = Detector operating in dual wavelength mode
6	Keypad Lock / Unlock	Open lock = Unrestricted keypad entry Closed lock = Parameter changes not allowed
4	Sticky Diagnostics	When the Absorbance screen displays the wrench icon, a sticky diagnostic is active. See <u>Section 5.2, User-Selected Diagnostics</u> , for a complete explanation of sticky diagnostics.
™★) ₽2	Local (Method #) / Remote Control	Local/Method number – If the detector is not controlled by a data system or other control device over the IEEE-488 bus, it displays a cursive "m" and the current method number or an asterisk (*) that indicates current conditions are not stored as a method. Remote/IEEE address – If the detector is controlled by a data system or other control device over the IEEE-488 bus, it displays a remote control icon containing the IEEE address for Channel A. In dual wavelength mode, the IEEE address for Channel B is the address displayed +1.
Numerical field	Run Time (Minutes)	Displays the time elapsed since you pressed the Run key, or since an inject start signal was received.

Table 3-1 Absorbance and Message Screen Icons (Continued)

Table 3-1 Absorbance and Message Screen Icons (Continued)

Icon or Field	Icon / Field Name	Function
21330>	Next	Indicates that pressing Next brings you to additional screens.
		Message screen icons (from left): Error, Question, Warning, Information, and Standby.

3.2.2 Using the Keypad

The 2487 Detector keypad (Figure 3-3) consists of 24 keys providing:

- Full numeric entry (10 digits plus a decimal point)
- Enter, Shift, CE (Clear Entry), Next, and Help functions
- s and t (used for navigation only; pressing s may also move you to the left, t to the right)
- A/B for channel selection
- Navigation to specific screens (HOME (absorbance), DIAGnostics, TRACE, CONFIGURE, METHOD, and SCAN)
- Primary function keys (Chart Mark, Auto Zero, and Run/Stop)
- Secondary function keys (Scale, Single or Dual Wavelength, Reset Clock, Lamp, Lock, Calibrate, System Information, Contrast, Previous, Cancel, +/–, and Clear Field)

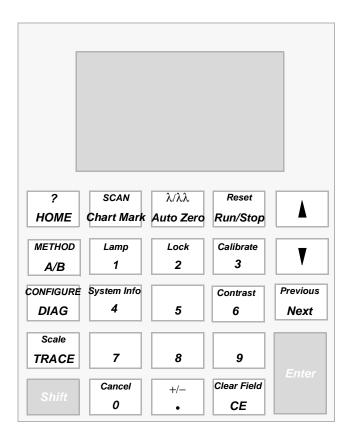


Figure 3-3 2487 Detector Keypad

Primary function keys take effect immediately, with no further entry required. Secondary function keys require you to enter information into parameter fields and to press Enter for the functions to take effect.

Keys that appear in all caps (**HOME**, **DIAG**, **TRACE**, **METHOD**, **CONFIGURE**, and **SCAN**) take you directly to a function from most screens.

For numerical entries from 1 to 9 on choice lists or menus, enter the number corresponding to the desired item, then press **Enter**. For the number 10, select **0**, then press **Enter**. To go to the end of a choice list, select •. For entries numbered 11 or 12, scroll to the desired item on the choice list, then press **Enter**.

<u>Table 3-2</u> explains the functions of the primary and secondary keys on the 2487 Detector keypad.

Key	Description				
Кеу	Unshifted Shifted				
? HOME	HOME – Displays the Absorbance screen containing the icons, and the Wavelength and AUFS fields.	? – Displays context-sensitive help when available.			
SCAN Chart Mark	Chart Mark – Causes a momentary pulse to the analog output (A and/or B, depending upon the current settings). This key has no effect if chart mark is disabled on both channels.	SCAN – Displays the list of options for generating and manipulating spectra.			
λλλ Auto Zero	Auto Zero – Sets the absorbance offset so that the analog output (A and/or B, depending on the current settings), reads 0 AU. This key has no effect if auto zero is disabled on both channels. You can enable or disable the auto zero function from the fourth Absorbance screen (Figure 3-4).	$\lambda/\lambda\lambda$ – When you are on the Absorbance screen, use this key to toggle between single and dual wavelength modes. The current mode is indicated by an icon on the display.			
Reset Run/Stop	Run/Stop – Starts or stops (freezes) the run clock. (The elapsed time appears near the lower right of the Absorbance screen.) Initiates scans.Reset – Resets the detector in clock to zero minutes. Returns detector to initial conditions for current method.				
	s, t – On screens with entry fields (e active field has a thick border. The a different field active. (s moves up or screens with a scrollable list, these the beginning of the list) or down (to have special instructions for the use the Display Contrast screen).	arrow keys can be used to make a left; t moves down or right.) On keys move the highlight up (toward oward the end). Other screens may			

Table 3-2 2487 Detector Keypad Description (Continued)

Kov	Description			
Кеу	Unshifted Shifted			
Previous Next	Next – Displays a screen with additional options related to the current screen. Repeated pressing of this key always brings you back to where you started. On most screens where this key is active, the NEXT arrow appears in the lower-right corner of the display.	Previous – When the Next key is available, Previous navigates through the screens in the reverse order.		
METHOD A/B				
CONFIGURE DIAG	DIAG – Displays the choice list of diagnostic routines.	CONFIGURE – Displays the first Configuration screen.		
Scale TRACE	TRACE – Displays the absorbance monitor trace for Channel A or B. Scale – When the waveled trace or spectrum screen visible, this function permit modification of the display in the X (time or waveleng Y (absorbance) dimension			
Shift	Shift – Enables the shifted function most keys). The shifted state is tem keystroke.			
0-9	0–9 – Enters the corresponding number into the current field. Also positions the cursor at the corresponding entry in a list (0 = tenth item). Selects the corresponding number from a choice list.	0–9 – See descriptions for specific shifted numeric keys.		

Key	Description		
Кеу	Unshifted	Shifted	
Lamp 1	1 – See 0–9 above.	Lamp – Displays the lamp use statistics for the currently installed lamp, and provides the ability to turn the lamp on or off. The current state of the lamp is indicated by an icon on the Absorbance screen.	
Lock 2	2 – See 0–9 above. Lock – When you are on the Absorbance screen, enable disables the keypad lock fear Use the lock to prevent inadvertent changes to deter settings. The current lock st indicated by an icon on the Absorbance screen.		
Calibrate 3	3 – See 0–9 above. Calibrate – Initiates the wavelength calibration rou		
System Info 4	4 – See 0–9 above.	System Info – Displays system information including software version, IEEE address, and instrument serial number.	
Contrast 6	6 – See 0-9 above.	Contrast – Permits adjustment of contrast (viewing angle) of the liquid crystal display.	
Cancel 0	0 – See 0–9 above.	Cancel – In some modes, Cancel backs out of a prompt without completing the task. The word "Cancel" appears as a cue in the lower-right border of the message text.	

Table 3-2 2487 Detector Keypad Description (Continued)

Table 3-2 2487 Detector Keypad Description (Continued)

Kov	Description		
Кеу	Unshifted	Shifted	
+/-	 Enters a decimal point. Also positions the cursor at the last entry in a list. 	+/ Some edit fields accept negative number entry. Use this function to invert the sign of the number in the active field.	
Clear Field CE	CE – Clears an editing change, and returns the contents of a field to its previous value. Sets the value to a unique word for some fields. For example, in the voltage offset diagnostic, you may enter either a numeric offset value or press CE to change it to OFF.	Clear Field – Blanks the current entry field before entering the desired values.	
Enter	Enter – Completes the entry in an edit field. Also advances the active field as if the Down arrow had been pressed (except after editing the wavelength on the Absorbance screen). Press Enter to acknowledge error messages and other prompts. In these cases, the word "Enter" appears as a cue in the lower-right border of the message text.		

3.2.3 Navigating the User Interface

When operating the 2487 Detector:

- 1. Press the Enter key or the s and t keys to navigate among editable fields on a display.
- 2. A thick border appears around the active field. Press Enter to advance the active field once you have completed entry.
- 3. When you make an error, press **CE** (Clear Entry) to undo any changes and return to the active entry field.
- 4. An active field containing a choice list is indicated by a number to the right of the field within the thick border. To display a choice list, press Enter. Then do one of the following:
 - Press the corresponding number key to select an item immediately.
 - Use the s and t keys to scroll through the list, then press Enter.

Note: If you know the number corresponding to the desired choice, you can press that number without pressing **Enter** first.

Note: The s and t keys do not increment or decrement numerical field entries. Use the numerical keypad.

Navigating to and from the Absorbance Screen

Pressing **HOME** brings you to the Absorbance screen from most screens. From the Absorbance screen, you can access several secondary functions. To move to the secondary function screens of the Absorbance screen, press **Next**. The secondary functions include:

- · Analog output specifications
- Filter type
- · Time constant
- · Absorbance offset
- · Voltage offset
- · Chart polarity
- Enable/disable inputs
- Enable/disable external events

Note: The parameters you enter into the secondary function fields become part of the current method conditions and are stored when you store the method (see <u>Section 3.3.6,</u> <u>Programming Timed Events, Threshold Events, and Methods</u>).

When in single wavelength mode, the detector displays three additional screens labeled 2 of 4, 3 of 4, and so on. When in dual wavelength mode, the detector displays four additional screens, labeled 2 of 5, 3 of 5, and so on (Figure 3-4).

Setting Up a Run

Once you press **HOME** to return to the Absorbance screen and select a wavelength mode $(\lambda \text{ or } \lambda \lambda)$, you are ready to set the detector up for a run. In addition to wavelength mode, you must program the following parameters before beginning a run:

- Operating wavelength
- Sensitivity
- Filter type
- Time constant
- Analog output sensitivity

Depending on other functions you may want to perform during a run, there are several other parameters you need to program. <u>Section 3.2.4</u>, <u>Primary and Secondary Functions</u>, and <u>Table 3-3</u> contain the function descriptions, fields, screen number, type of function, display units, allowable ranges, and the default settings for the Absorbance screen and the secondary function screens, for both single and dual wavelength operation.

3.2.4 Primary and Secondary Functions

From the Absorbance screen, you can access the following functions, either from the Absorbance screen or by pressing the **Next** key:

Wavelength – Defines the operating wavelength for the channel.

AUFS (absorbance units full scale) – Defines the relationship between the absorbance and the output voltage. The output voltage reaches full scale (2 V or 10 mV) when the current absorbance attains the AUFS value.



Attention: Changing the sensitivity (AUFS) setting affects both the 2 V and 10 mV outputs.

Filter type - Defines your choice of noise filter (Hamming filter is the default).

Analog out (single λ **)** – You can chart out the analog connections for the following parameters:

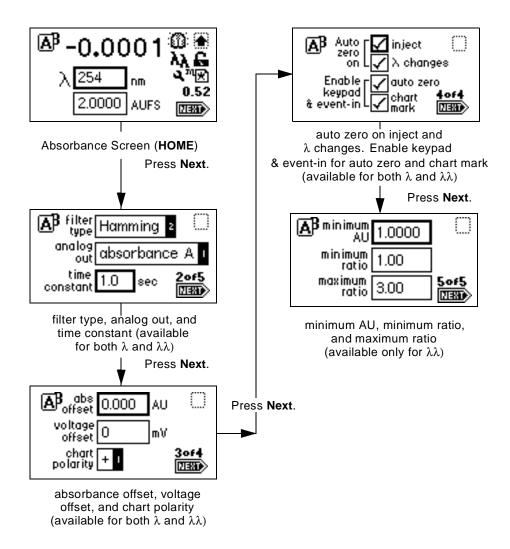
 Absorbance – Charts the current absorbance out the selected channel analog output connectors (2 V and 10 mV) scaled to the AUFS setting for that channel, and adjusted based on the voltage and absorbance offsets for that channel. For the 2 V output with 0 used for both the voltage and absorbance offsets:

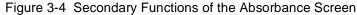
Volts out = Absorbance * 2 V/AUFS

 Log absorbance – Charts small and large peaks on a single chromatographic output. In this mode, voltage offset is used but absorbance offset is ignored. For the 2 V output:

Volts out = $(Log_{10} [Absorbance + 1] * 2 V) + voltage offset$

- **Sample energy** Charts the lamp energy from the sample side of the flow cell. Sample energy scaling is fixed at 150 nA/V.
- **Reference energy** Charts the lamp energy from the reference side of the flow cell. Reference energy scaling is fixed at 150 nA/V.





Analog out (dual λ) – In addition to the selections for single λ above, you can chart the same parameters on the other channel at a different wavelength, and you can chart the following parameters:

 MaxPlot – Charts the absorbance of multiple compounds with different absorbances at two different wavelength on a single data channel. Scaling for MaxPlot is the same as for Absorbance above, except that the charted absorbance is the larger of the two absorbances measured on the wavelengths on Channel A and Channel B. The 2487 Detector uses the AUFS, absorbance offset, and voltage offset of the selected channel regardless of which absorbance (Channel A or Channel B) is larger.

Volts out = Larger absorbance (A or B) * 2 V/AUFS (of selected channel)

- RatioPlot (A/B and B/A) Charts the ratio of absorbances at two wavelengths. Theoretically, the ratio is constant for a pure chromatographic peak and variable for an impure peak. This mode uses the three ratio parameters on screen 5 of 5 (Figure 3-4).
 - Minimum AU: This setting defines the minimum AU value for either wavelength (A or B) before the actual ratio is calculated. Both absorbance values (A & B) must be above this value or 0 V is charted. If both absorbances are above this value, the absorbances are divided (either A/B or B/A) and charted. The output voltage is scaled proportionally to the ratio depending on the selected channel minimum ratio and maximum ratio settings.
 - Minimum ratio: An actual ratio equal to the minimum ratio value results in 0 V being charted.
 - Maximum ratio: An actual ratio equal to the maximum ratio results in a full scale output of 2 V. Absorbance offset is ignored with this selection.

For a RatioPlot, the actual voltage charted is:

Volts out = 0 V if Absorbance A and B < minimum AU

Volts out = (Absorbance ratio – minimum ratio) * 2 V / (maximum ratio – minimum ratio)

To ensure proper operation of the RatioPlot function, be sure the selected time constants for both channels are set to the same value.

• Difference Plot (A-B and B-A) – Charts the difference in absorbances at two different wavelengths. The scaling for the difference plot is identical to the Absorbance selection above, except that the charted absorbance is the difference (subtracted) in value of the two absorbances measured on the A and B

wavelengths. The 2487 Detector uses the AUFS, absorbance offset, and voltage offset of the selected channel for scaling.

Volts out = Absorbance difference (A-B or B-A) * 2 V / AUFS (of selected channel)

Time constant – Adjusts the noise filter (time constant) to achieve the optimum signal-to-noise ratio without changing the sensitivity setting. See the discussion <u>"Filtering Noise"</u> in <u>Section 1.2.3</u>, <u>Waters TaperSlit Flow Cell</u>, for more information.

Absorbance offset – Provides an offset in absorbance for adjusting the analog output. The default setting of 0.0 for absorbance offset charts 0.0 AU at 0.0 V. An absorbance offset of 1.0000 offsets the charted absorbance by 1.0000 AU. An absorbance offset of 1.0000 and an AUFS setting of 2.000 results in a –1.0000 AU reading charted at 0.0 V. This allows 0 AU to be charted at midscale of a chart recording allowing for a full 1.0 AU range in both the positive and negative direction. For the 2 V output:

Voltage adjusted = (Absorbance offset / AUFS) * 2 V

Voltage offset – Adjusts the charted analog output signal. Entered in millivolts, voltage offset adjusts the 2 V signal by the entered value. This is useful for making minor adjustments, and for nulling any offset between the 2487 Detector and a connected external data system. The voltage offset also affects the 10 mV output by 1/200 of the entered value.

Chart polarity – Inverts the charted chromatogram. Entering the plus sign (+) produces a normal chromatogram; entering the minus sign (–) produces an inverted chromatogram.

Auto zero on inject – Selected by default, this parameter results in an auto zero each time an inject start signal is received by the 2487 Detector via contact closure, IEEE-488, or through the front panel. You can disable this parameter by pressing any numerical key to deselect this box for either or both channels.

Auto zero on λ changes – Selected by default, this parameter results in an auto zero each time a wavelength change is requested. We strongly recommend that you allow this parameter to remain selected except when performing specific advanced operations. If you deselect this function, significant changes in measured absorbance may occur after each wavelength change. You can disable this parameter by pressing any numerical key to deselect this box for either or both channels.

Enable keypad & event-in auto zero – Selected by default, this parameter results in an auto zero each time an auto zero is requested via contact closure or through the front panel. You can disable this parameter by pressing any numerical key to deselect this box for either or both channels.

Enable keypad & event-in chart mark – Selected by default, this parameter results in a chart mark each time one is requested via contact closure or through the front panel. You can disable this parameter by pressing any numerical key to deselect this box for either or both channels. This function is channel-specific: one channel can be used for annotation while the other channel is unaffected by a chart mark request.

Function	Screen	Туре	Units	Range	Default
λ (wavelength)	1 (Absorbanc e screen)	numeric	nm	integer 190 nm to 700 nm	254 nm
AUFS	1	numeric	AUFS	0.0001 to 4.0000	2.0000
filter type	2 (of 4 or 5)	choice	none	Hamming RC None	Hamming
analog out (single λ)	2 (of 4)	choice	none	absorbance A log abs A sample energy ref energy	absorbance A
analog out (dual λ)	2 (of 5)	choice	none	absorbance A log abs A absorbance B log abs B maxplot A, B difference A-B difference B-A ratio A/B ratio B/A A samp energy A ref energy B samp energy B ref energy	absorbance A
time constant	2 (of 4 or 5)	numeric	Sec	Hamming (λ) : 0.1 to 5.0 Hamming $(\lambda\lambda)$: 1 to 50 RC: 1 to 99 0 to disable filtering	1.0
abs offset	3 (of 4 or 5)	numeric	AU	-4.000 to +4.000	0.000

Table 3-3	Primary and	Secondary Function	n (Method) Parameters
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 Table 3-3 Primary and Secondary Function (Method) Parameters (Continued)

Function	Screen	Туре	Units	Range	Default
voltage offset	3 (of 4 or 5)	numeric	mV	integer –2000 to 2000	0
chart polarity	3 (of 4 or 5)	choice	none	+ _	+
Auto zero on inject	4 (of 4 or 5)	check box	none	Checked/not checked	Checked
Auto zero on λ changes	4 (of 4 or 5)	check box	none	Checked/not checked	Checked
Enable keypad & event-in auto zero	4 (of 4 or 5)	check box	none	Checked/not checked	Checked
Enable keypad & event-in chart mark	4 (of 4 or 5)	check box	none	Checked/not checked	Checked
minimum AU	5 (of 5)	numeric	AU	0.0001 to 4.0000	0.1000
minimum ratio	5 (of 5)	numeric	none	0.00 to 999.99	0.00
maximum ratio	5 (of 5)	numeric	none	0.00 to 999.99	2.00

3.2.5 Operating the Trace and Scale Functions

The Trace function allows you to display an absorbance trace for the last *n* minutes (up to 60) of detector operation.

- When you press the **TRACE** key, the 2487 Detector displays the absorbance acquired over the last 30 minutes by default. It updates the trace once every 20 seconds.
- When you press the Scale key (Shift TRACE), the detector displays the scaled trace with T1 (ending time) displayed (-30 for the last 30 minutes) by default.

You can change the ending time parameter to any number from 1 to 60. You can use the Scale function to "zoom" in on a particular section of the trace.

To display the Scale parameters after you press the Scale key:

- 1. Press Next to display T2 (starting time). The default is 0.
- 2. Press Next again to display AU1 (starting or low absorbance). The default is auto.
- Press Next again to display AU2 (ending or high absorbance). The default is auto.

By entering appropriate times and absorbance numbers in the four scaling parameter boxes, you can zoom in on one section of the current absorbance trace.

- For AU1 and AU2, press CE to reset to auto.
- **T1** represents the left side of the trace, or ending time to be displayed (default is -30).
- T2 represents the right side of the trace, or starting time (default is 0).

<u>Figure 3-5</u> shows a 60-minute trace of continuous injections of caffeine and propyl paraben in 50% MeOH/50% H₂O.

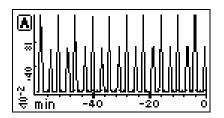


Figure 3-5 Scaled Trace of Continuous Injections with T1 Changed to -60

<u>Figure 3-6</u> shows a 5-minute scaled trace (or zoom) of the 60 minutes of continuous injections shown in <u>Figure 3-5</u>. T1 is changed to –5. T2 is changed to 0. AU1 and AU2 remain as "auto."

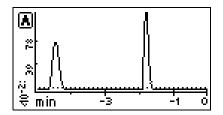


Figure 3-6 Scaled Trace for 5 Minutes of Continuous Injections with T1 Changed to -5

<u>Figure 3-7</u> shows a 60-minute scaled trace similar to that shown in <u>Figure 3-5</u> with the starting absorbance or AU1 changed from auto to 1. T1 remains at 60. T2 remains at 0.

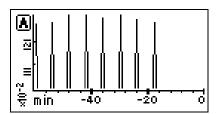


Figure 3-7 Scaled Trace for 60 Minutes of Continuous Injections with AU1 Changed to 1

<u>Figure 3-8</u> shows a 60-minute trace on Channel B scaled to the last 45 minutes. T1 is changed to -45.

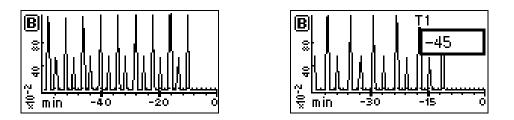


Figure 3-8 Scaled Trace Changing T1 to -45

As you modify the output using the Scale function, the Trace function continues to display the 2487 Detector output in real time on either or both channels.

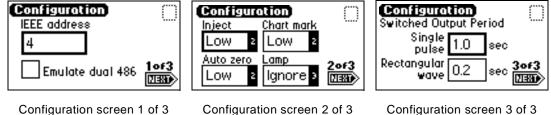
3.2.6 Operating Other Detector Functions

Configuring the 2487 Detector

The 2487 Detector is configured at IEEE-488 remote address 4 by default. To change the default, you need to access the Configuration screens.

Press the CONFIGURE key (Shift DIAG). The first of three Configuration screens appears (Figure 3-9).

Note: Other functions, such as specifying event inputs and enabling pulse periods, are stored within the Configuration screens.



Configuration screen 3 of 3

Figure 3-9 Configuration Screens

Attention: To prevent acquisition of incorrect data when operating the 2487 Detector in STOP dual wavelength mode under the control of the Millennium³² software or a PowerLine system, you must select a data sampling rate of 1 point per second.

Note: When the 2487 Detector is operating in dual wavelength mode and connected to a PowerLine controller or a 2690 Alliance Separations Module version 1.15 or lower using the IEEE-488 communications bus, you must enable the Emulate dual 486 configuration option.

The IEEE-488 address you assign to the detector must be an even number from 2 to 28 (the detector assigns the next consecutive address to the "second" 486 Detector).

Note: When the 2487 Detector is connected to the Millennium³² software, you do not need to select the Emulate dual 486 option.

Setting the IEEE Address

There are two editable entry fields on the first Configuration screen to set the IEEE address:

- IEEE address Enter the detector's IEEE-488 bus address, from 2 through 29. If you press the CE key, the 2487 Detector is removed from IEEE control. The default IEEE address is 4.
- Emulate dual 486 This option allows the 2487 Detector to appear on your external controller as two Waters 486 Detectors. This option provides for control and data acquisition of the 2487 Detector's dual wavelength capabilities on older Waters systems, such as Millennium³² software, PowerLine controllers, and some versions of the 2690 Separations Module.

Note: When using the *Emulate dual 486* option, the 2487 Detector acts as a 486 Detector. See page 58 in Chapter 2.

If you select the **Emulate dual 486** configuration option and operate the detector in dual wavelength mode on one of these systems, the detector displays two addresses on the first Configuration screen: the defined IEEE address (see the Note below) and the next consecutive address.

Pressing any numerical key toggles the Emulate dual 486 check box.

Note: When operating in dual wavelength mode, if you select the **Emulate dual 486** option, you can define only even-numbered IEEE addresses. The next consecutive IEEE address (that is, the odd number that follows the even number you defined) is assigned to the "second" 486 Detector.

Configuring Event Inputs (Contact Closures)

You also use the **CONFIGURE** key to edit event input settings and specify switched output settings.

There are four editable entry fields on the second Configuration screen (Figure 3-9):

- **Inject** You can specify the Inject input to signal the start of a run. This event resets the run-time clock and applies initial method conditions immediately:
 - High Start run when contact closure changes from off (open) to on (closed).
 - Low Start run when contact closure changes from on (closed) to off (open).
 - Ignore Do not respond to Inject start input.

Use the Enter key and the numeric keypad or the s and t keys to select the appropriate entry.

 Chart mark – You can specify the Chart mark input to create a chart mark on Channel A and/or Channel B. You determine the response of the channel using the enable chart mark function explained in <u>Table 3-3</u> and shown in <u>Figure 3-4</u>.

 High – Create chart mark(s) when contact closure changes from off (open) to on (closed).

- Low - Create chart mark(s) when contact closure changes from on (closed) to off (open).

- Ignore - Do not respond to Chart mark input.

Use the Enter key and the numeric keypad or the s and t keys to select the appropriate entry.

• Auto zero – You can configure the Auto zero input to auto zero absorbance readings on Channel A and/or Channel B. You determine the response of the channel using the enable auto zero function explained in <u>Table 3-3</u> and shown in <u>Figure 3-4</u>.

High – Auto zero the channel when contact closure changes from off (open) to on (closed).

 Low – Auto zero the channel when contact closure changes from on (closed) to off (open).

- Ignore - Do not respond to Auto zero input.

Use the Enter key and the numeric keypad or the s and t keys to select the appropriate entry.

- Lamp You can configure the Lamp input level to turn the deuterium lamp on or off from an external device as follows:
 - High Turn lamp on when contact closure is on (closed).
 - Low Turn lamp on when contact closure is off (open).
 - Ignore Do not respond to Lamp input.

Use the **Enter** key and the numeric keypad or the s and t keys to select the appropriate entry.

The default for Inject, Chart Mark, and Auto Zero is Low; the default for Lamp is Ignore.

Setting Pulse Periods

You use the third Configuration screen (Figure 3-9) to set pulse or signal width or to activate a pulse or rectangular wave on SW1 or SW2.

• Single pulse (in seconds) – If SW1 or SW2 is programmed to generate a pulse as a timed or threshold event, then the period of the signal (single pulse width) is as specified in this field (range is 0.1 to 60 seconds).

• **Rectangular wave (in seconds)** – If SW1 or SW2 is programmed to initiate a rectangular wave as a timed or threshold event, then the period of the signal (the width of one pulse period in a rectangular wave or pulse train) is as specified in this field (range is 0.2 to 60 seconds).

Figure 3-10 shows the difference between a single pulse and a rectangular wave.

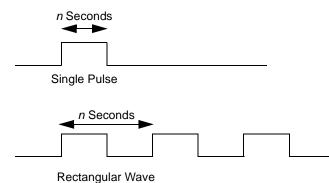


Figure 3-10 Setting the Pulse Period or Signal Width Using SW1 or SW2

Setting Display Contrast

The Contrast function allows you to adjust the contrast of the 2487 Detector display screen. When you press the Contrast key (Shift 6), the Display Contrast screen appears (Figure 3-11).

Display Contrast
Use the 🔺 and 🔻
keys to adjust display
contrast. Enter

Figure 3-11 Display Contrast Screen

Use the s and t keys to adjust the contrast of the display.

Displaying System Info

The **System Info** key (**Shift 4**) displays information about the 2487 Detector, including the serial number, the software version number, and the IEEE address, if applicable (Figure 3-12).

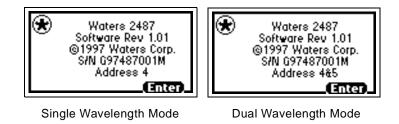


Figure 3-12 System Info Screen Examples

Using Help

The 2487 Detector has limited context-sensitive help. When you press ? (Shift HOME) from a point in the program that has a Help screen associated with it, the screen is displayed (Figure 3-13).

Enter the desired wavelength for detection on channel A. Range: between 190 and 700.

Figure 3-13 Help Screen

To dismiss the Help screen, press **Enter**. If help is not available for the function you are working on, pressing **?** gets no response.

3.3.1 Overview of Detector Operation

Note: If you are operating the 2487 Detector under the control of an external system, you can program any parameters not controlled by the external data system at the front panel of the detector before the external system takes control.

Note: To prevent reabsorption of dissolved oxygen, we recommend that you run the solvent degasser continuously when operating the 2487 Detector at wavelengths less than 230 nm.



Attention: To maintain optimum system performance, be sure to replace the left front panel cover before resuming normal operation of the 2487 Detector.

Operating Modes

You can use the 2487 Detector in either single or dual wavelength mode over a range of 190 to 700 nm. The detector defaults to the mode of operation when power to the instrument was last shut off.

When the detector is operating in single wavelength mode, you can configure analog outputs on Channel B. In single wavelength mode, the detector tracks a single wavelength on both Channel A and B. You can use Channel B to:

- Track absorbance at an alternate AUFS
- · Monitor sample or reference energy outputs
- · Set a different time constant

See <u>Table 3-3</u> for more information on operating parameters in either single or dual wavelength mode. When the 2487 Detector is under the control of older Waters data systems, it functions as a Waters 486 Detector (see the discussion <u>"Remote Control"</u>).

Stand-Alone Operation

When using the 2487 Detector as a stand-alone instrument, you can store up to 10 methods containing up to 16 timed and 2 threshold events each. An asterisk in the method number field on the Absorbance screen indicates current conditions, not a stored method. See <u>Section 3.3.6</u>, <u>Programming Timed Events</u>, <u>Threshold Events</u>, and <u>Methods</u>, for information on how to store a method.

Remote Control

To operate as part of a Waters Alliance system or under Millennium³² control, the 2487 Detector uses the IEEE-488 bus connector (see <u>Section 2.6.3, Making IEEE-488</u> <u>Connections in Breeze and Millennium³²</u>).

Under the control of the Millennium Chromatography Manager version 2.15 or lower, a 2690 Alliance Separations Module version 1.15, or a PowerLine controller, the 2487 Detector appears on the data system configuration screen or on the busLAC/E as two 486 Detectors if:

- The **Emulate dual 486** option is enabled and the detector is operating in dual wavelength mode.
- The detector is operating in dual wavelength mode.
- The IEEE address is an even-numbered address from 2 to 28 (Figure 3-9). The detector assigns the next consecutive address to the "second" 486 Detector.

Note: When the 2487 Detector is connected to the Millennium³² software versions 3.0 and higher, you do not need to select the **Emulate dual 486** option.

To connect the 2487 Detector to your HPLC system, see <u>Section 2.4, Making Fluidic</u> <u>Connections</u>.

When under the control of an external data system, the 2487 Detector is operating under remote control conditions. The Remote Control icon, containing the IEEE-488 address, is present on the Absorbance screen (see Figure 3-2 and Table 3-1).

See <u>Section 2.6, Making Signal Connections</u>, for more information on connecting the 2487 Detector to an external system.

3.3.2 Verifying the Detector

After you have installed the 2487 Detector, verify that the detector is operating properly by performing the procedures in this section.

This section describes how to perform certain tests needed to verify your system. When you complete these procedures successfully, the 2487 Detector optics and electronics are functioning properly. For complete validation procedures, you must obtain the Waters accuracy and linearity cuvette kits and Qualification Workbooks for the 2487 Detector. See <u>Appendix B</u>, <u>Spare Parts</u>, for part numbers and ordering information.

Note: Before you pump solvent or mobile phase through the system, flush the lines with filtered, degassed, and sparged HPLC-grade methanol. Then pump your mobile phase, provided that there are no miscibility problems, at 1 mL/min for 15 minutes minimum.

Before You Begin

Since the 2487 Detector is shipped dry, you must pump solvent through the unit before initial use.

Note: To ensure accurate verification, be sure to start up the 2487 Detector and follow steps 1 to 3 in this section and 1 to 4 in <u>"Recording Sample and Reference</u> <u>Beam Energies</u>" before pumping any mobile phase or solvent through the flow cell.

- 1. Connect the detector to a data system or chart recorder. Refer to <u>Chapter 2,</u> <u>Installing the 2487 Detector</u>, for complete information on connecting the 2487 Detector to external devices.
- Turn on the Waters 2487 Detector. The front panel displays a series of initialization messages for about five minutes. Refer to <u>Section 3.1, Starting Up</u> <u>the 2487 Detector</u>.

When initialization is complete, the 2487 Detector displays the Absorbance screen (Figure 3-2). For an explanation of the 2487 Detector keypad, see <u>Section 3.2.2, Using the Keypad</u>.

3. Allow the detector to warm up for at least 30 minutes before operating.

If the startup verification diagnostics fail, note the error message to determine the corrective action. Refer to <u>Chapter 5, Error Messages, Diagnostics, and</u><u>Troubleshooting</u>.

Recording Sample and Reference Beam Energies

To determine baseline values on your 2487 Detector for future reference and to monitor lamp aging (decreased lamp output energy), you need to record the baseline sample and reference beam energies to compare against future readings. Use these baseline values to troubleshoot the 2487 Detector to determine whether the:

- Solvent is contaminated
- Flow cell is contaminated
- Lamp needs to be replaced
- Air bubble in flow cell

To record the sample and reference beam energies:

1. Set the 2487 Detector wavelength to 230 nm by entering 230 into the λ field on the Absorbance screen when the field is active (surrounded by a thick border), then press Enter.

 Press DIAG 2, Sample & ref energy, and record the sample and reference energies for 230 nm for later comparison. The Sample and Reference Energy Diagnostic display appears (Figure 3-14).

<mark>Α</mark> β Energy	Sample: ()
λ	12.046 nA
254 nm	Ref: 21.537 nA

Figure 3-14 Sample and Reference Energy Diagnostic Display

3. Record the numbers for later comparison.

Note: Always run this procedure each time you change the 2487 Detector lamp.

4. Flush the flow cell with approximately 30 to 60 mL of HPLC-grade methanol at a flow rate of 1 mL/min for 15 minutes minimum to clean the cell.

You are now ready to run the peak response verification test. If the following test fails, repeat the it.

Make the fluid connections described in <u>Section 2.4, Making Fluidic Connections</u>, before beginning the peak response test.

Verifying Peak Response

This test checks the 2487 Detector peak response. To check peak response:

- 1. Set the wavelength to 254 nm by entering **254** into the λ field on the Absorbance screen when the field is active, then press **Enter**.
- 2. Press Enter again to activate the sensitivity field.
- 3. Set the sensitivity to 2.0 AUFS.
- 4. Set the pump flow rate in your HPLC system to 1.0 mL/min.
- 5. Inject 1 µL acetone.

When the test is successful, your chart recorder or data system displays a peak.

3.3.3 Wavelength Calibration

The 2487 Detector offers you the option of calibrating the detector manually from the keypad. You can use the manual calibration key to recalibrate the detector at any time during operation, or if there are any calibration errors during startup. You do not have to reboot the detector after a successful wavelength calibration.

To calibrate the detector manually:

1. Press Calibrate (Shift 3) from the 2487 Detector keypad.

A message appears asking whether you removed the cuvette and flushed the flow cell with a transparent (methanol or water are recommended) solvent (Figure 3-15).

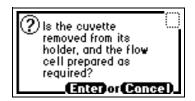


Figure 3-15 Cuvette Wavelength Calibration Message

2. Press Enter to continue the calibration cycle, or press Cancel to return to the Absorbance screen without calibrating the detector.

After you press **Enter**, the detector cycles through the calibration procedure and briefly displays a series of initialization messages similar to those you saw at startup (see <u>Section 3.1.1, Initializing the 2487 Detector</u>).

If calibration is successful, the detector beeps three times and displays the maximum error in nanometers of the furthest calibration shift from the last calibration (Figure 3-16).

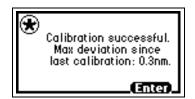


Figure 3-16 Calibration Successful Message

3. Press Enter to complete calibration.

A "Calibration complete" message is displayed momentarily. Other messages such as "Optimizing system performance" and "Restoring last setup" may appear before the display returns to the Absorbance screen.

Note: If calibration is successful, the error message (<Error>) that was displayed on the Absorbance screen before you recalibrated the detector disappears.

4. If calibration is not successful, retry, recycle power to the detector, or see <u>Chapter 5, Error Messages, Diagnostics, and Troubleshooting</u>.

3.3.4 Operating the Detector in Single Wavelength Mode

The 2487 Detector is optimized for single wavelength operation, which is the default operating mode. To invoke single wavelength mode:

1. From the Absorbance (or HOME) screen, press the $\lambda/\lambda\lambda$ key (Shift Auto Zero) if the detector is in dual wavelength (the wavelength icon shows $\lambda\lambda$) mode. The detector displays a message indicating it is switching to single wavelength operation (Figure 3-17).



Figure 3-17 Wavelength Mode Change Message (for Single Wavelength)

2. Enter the wavelength and sensitivity on the Absorbance screen and any secondary parameters, timed, or threshold events (Figure 3-4 and Table 3-3 through Table 3-6).



Attention: Changing the sensitivity (AUFS) setting affects both the 2 V and 10 mV outputs.

3. To select a second sensitivity setting while in single wavelength mode, press A/B and enter the appropriate AUFS on the Channel B screen.

A single wavelength is tracked on Channel A, allowing you to use Channel B to monitor absorbance with an alternate AUFS setting, or to use the LOGABS setting on Channel B while making the primary absorbance measurement using a specific AUFS on Channel A. For example, while operating in single wavelength mode, you can set an AUFS of 2.0000 on the second channel, providing 1.000 V/AU on the Channel B 2 V output.

The detector automatically engages the second order filter for all wavelengths \geq 370 nm.

3.3.5 Operating the Detector in Dual Wavelength Mode

You can operate the 2487 Detector with expanded chart out selections using dual wavelength mode. In addition to Log Absorbance and Absorbance offered with single wavelength mode, dual wavelength offers the following functions:

- Absorbance (A and B)
- Log absorbance (A and B)
- Sample energy (A and B)
- Reference energy (A and B)
- MaxPlot
- RatioPlot (A/B and B/A)
- Difference plot (A-B and B-A)

<u>Section 3.2.4, Primary and Secondary Functions</u>, and <u>Table 3-3</u> explain these functions and their default and operating parameters in detail.

Note: When the 2487 Detector is operating in dual wavelength mode and connected to a PowerLine controller, the Millennium Chromatography Manager version 2.15 or lower or the Waters Alliance 2690 Separations Module version 1.15 or lower using the IEEE-488 communications bus, you must enable the **Emulate dual 486** configuration option (see the discussion <u>"Configuring the 2487 Detector"</u> in <u>Section 3.2.6</u>, <u>Operating Other Detector Functions</u>).

Changing from Single to Dual Wavelength Mode

To change from single to dual wavelength mode:

1. From the Absorbance (or HOME) screen when in single wavelength mode (the wavelength icon shows λ), press the $\lambda/\lambda\lambda$ key (Shift Auto Zero). This key toggles between single and dual wavelength modes. A momentary message ("Setting up dual wavelength mode") appears (Figure 3-18).

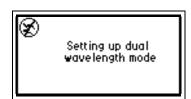


Figure 3-18 Wavelength Mode Change Message (for Dual Wavelength)

- 2. Enter the wavelength to be monitored in the λ field, then press Enter.
- 3. Enter the other operating parameters and any timed or threshold events, if desired.
- 4. Press the A/B key to switch channels. The Absorbance screen for the other channel appears.
- 5. Enter the operating parameters for the second wavelength to be monitored, including any timed and threshold events, if desired.

For more information on operating the detector in single wavelength mode, see <u>Section 3.3.4</u>, <u>Operating the Detector in Single Wavelength Mode</u>. For more information on programming timed and threshold events, see <u>Section 3.3.6</u>, <u>Programming Timed</u> <u>Events</u>, <u>Threshold Events</u>, and <u>Methods</u>.

STOP

Attention: In dual wavelength mode:

- If both selected wavelengths are greater than 370 nm (+/- 1 nm), the detector applies the second order filter to block unwanted UV light.
- If both selected wavelengths are less than 370 nm (+/- 1 nm), the detector removes the second order filter.

• If the selected wavelengths bracket the 370 nm (+/- 1 nm) threshold, the detector does not apply the second order filter and issues a warning message that any data collected for the wavelength above 370 nm may contain inaccuracies because of possible UV light interference (second order effects).

We recommend that, when in dual wavelength mode, you select a wavelength pair either below or above 370 nm. If one or both of the selected wavelengths spans the 370 nm threshold, the 2487 Detector beeps three times and issues the warning message shown in <u>Figure 3-19</u>. Because of possible UV light interference (second order effects), you may observe additional peaks and inaccurate peak areas.



Figure 3-19 Dual Wavelength 370 nm Threshold Warning Message

Obtaining a RatioPlot

The RatioPlot output, on one channel only (Channel A), depends on the entries you make in the minimum ratio and maximum ratio parameters on Absorbance screen 5. You must be operating the 2487 Detector in dual wavelength mode to obtain a ratio plot. The RatioPlot provides a plot of the ratio of the absorbances of two wavelengths from 0 V to 2 V (10 mV). The minimum and maximum ratio parameters are measured ratio units, not absorbances. See <u>Section 3.2.4</u>, <u>Primary and Secondary Functions</u>, for a complete explanation of the RatioPlot function.

To obtain a RatioPlot:

- 1. Be sure the 2487 Detector is operating in dual wavelength mode (see the previous discussion <u>"Changing from Single to Dual Wavelength Mode"</u>).
- 2. From the Absorbance screen, press Next to reach screen 2 of 5 (Figure 3-4).
- 3. Choose the filter type (Hamming is the default) in the first field, then press Enter.
- 4. In the second field (**analog out**), press **8**, ratio A/B, or **9**, ratio B/A, depending on which ratio value you want to monitor.
- 5. Press Enter to select the ratio plot.
- 6. Press Next until you reach screen 5 of 5.
- 7. Enter the minimum AU, then press Enter.

Note: The minimum AU field contains a threshold value. The RatioPlot function does not plot if either wavelength does not exceed the minimum AU threshold.

8. Enter the minimum ratio for the RatioPlot, then press Enter.

- 9. Enter the maximum ratio for the RatioPlot, then press Enter.
- 10. Return to the Absorbance screen by pressing the **HOME** key.

Obtaining a MaxPlot

The 2487 Detector allows you to obtain a MaxPlot by monitoring absorbance at two selected wavelengths, while plotting the maximum absorbance for each sample component. To run a scan using the MaxPlot function:

- Be sure the 2487 Detector is operating in dual wavelength mode (see the previous discussion <u>"Changing from Single to Dual Wavelength Mode</u>").
- From the Absorbance screen, press Next to reach screen 2 of 5 (Figure 3-4).
- Choose the filter type (Hamming is the default) in the first field, and press Enter.
- 4. In the second field (analog out), press 5, maxplot A,B.
- 5. Press Enter to select the MaxPlot function.
- 6. Return to the Absorbance screen by pressing the HOME key.

3.3.6 Programming Timed Events, Threshold Events, and Methods

Introduction

The 2487 Detector allows the storage and retrieval of up to 10 methods. The detector references stored methods by the numbers 1 to 10. An asterisk in the method number icon (<u>Table 3-1</u>) tells you the current conditions are not stored. If you are operating using a stored method, the method number is displayed on the Absorbance screen (<u>Figure 3-2</u>).

If you edit a parameter such as wavelength or AUFS, you are editing the current conditions (Method *) which can be stored as a method. You may store the method in one of the 10 available method storage slots, or you can replace the current method with one of the methods previously stored. When you recall a previously stored method, you replace the existing method conditions with those of the stored method.

The method number displayed on the Absorbance screen is that of the retrieved method until you make a change. Any parameter change (for example, wavelength or AUFS) alters the current conditions so that the original recalled method is no longer in effect, causing the method number to change to an asterisk.

The operating parameters at the time of system shutdown are restored; however, any timed events or thresholds associated with the method are deactivated when power is restored. On startup, you always see an asterisk inside the method icon on the Absorbance screen.

When the 2487 Detector is operating under remote control, that is, control by the Millennium³² software, the PowerLine controller, or an Alliance Separations Module, the remote icon (<u>Table 3-1</u>) containing an IEEE-488 address is displayed.

Timed Events

You can program up to 16 timed events to the nearest 0.01 minute. As you enter timed events, each new event is appended to the end of the timed event list. If you enter a time that is not in sequence with the events entered previously, the timed event list is sorted automatically when you press **Next**. The 2487 Detector allows programming of the timed events shown in <u>Table 3-4</u>.



Attention: Changing the sensitivity (AUFS) setting affects both the 2 V and 10 mV outputs. For example, 1 AU on a 2 V output gives 0.5 AU/V and 2 AU on a 2 V output gives 1 AU/V.

Number	Event	Units	Range or Default	Specify Channel
1.	wavelength	nm	190 to 700	yes
2.	time constant	seconds	$\begin{array}{llllllllllllllllllllllllllllllllllll$	yes
3.	sensitivity	AUFS	0.0001 to 4.0000	yes
4.	chart mark (10% of full scale)	does not apply	does not apply	yes
5.	polarity	1. + 2. –	+	yes
6.	auto zero	does not apply	does not apply	yes
7.	lamp	1. off 2. on	off	No

Table 3-4 Timed Event Parameters

Number	Event	Units	Range or Default	Specify Channel
8.	switch 1	1. high 2. low 3. pulse 4. rect wave	high	no
9.	switch 2	1. high 2. low 3. pulse 4. rect wave	high	no
10.	threshold	AU	-4.0000 to 4.0000 or variable, depending on output selection	yes

Table 3-4 Timed Event Parameters (Continued)

To program a new timed event:

1. Press the **METHOD** (Shift A/B) key on the 2487 Detector keypad. The Method choice list is displayed (Figure 3-20).



Figure 3-20 Method Choice List

2. Press 1, Timed events. An active field for entering the time of the event is displayed.

3. Enter the time for the event. When you begin entering the time, additional fields are displayed (Figure 3-21).

GN	Event 1 of 1	\cap
Ât	25.00 min	™ X
Set	wavelength	1
То	254 nm	DIEST

Figure 3-21 Timed Events Screen

- Press Enter to enter the time. To advance to the Set field (Events choice list), press the t key.
- 5. Press Enter again to display the choice list, or, if you know the event number, press the number for the event you are programming (<u>Table 3-4</u>).
- Enter the appropriate swavelength in nm into the **To** field, if the **To** field is displayed.

Note: If you want the same event programmed on both channels, you must enter two events, one for Channel A and one for Channel B.

7. Press A/B to set the threshold on the other channel.

ON A or **ON B** indicates the channel the event is programmed on. You can program all events on Channel A, all on Channel B, or some on Channel A and some on Channel B. Event programming is time-based, not channel-specific.

8. Press Next to advance to a new timed event.

To delete a timed event, press **CE** when the time field is active to change it to OFF.

- Press HOME to return to the Absorbance screen, then press Run/Stop to start the method.
- 10. Press Reset to reset the run clock to 0.

If the 2487 Detector is under the control of the Waters 717plus Autosampler or another external device, the inject start programmed from that device runs the method.

Note: If you are working in real time under current conditions (method *), at a power failure or shutdown you lose all timed or threshold events if you do not store them as a method. See the discussion <u>"Storing a Method"</u> in this section.

Threshold Events

You can program threshold events on both Channel A and Channel B to control the switch contact closure outputs, for example, when using a fraction collector. You can program the switch to change when the programmed output (absorbance, ratio, energy, etc.) on the 2487 Detector Channel A or B is above a specified threshold. Below the specified threshold, the switch is set as shown in <u>Table 3-6</u>.

You can program the contact closure switches as shown in Table 3-5.

Table 3-5	Threshold Events "Set" Parameters	
-----------	-----------------------------------	--

Number	Event		
1.	set switch 1		
2.	set switch 2		

You can program the switch parameters as shown in Table 3-6.

Table 3-6 Threshold Events "To" Parameters

Number	Set To	Below Threshold Switch State
1.	on	off
2.	off	on
3.	pulse	off
4.	rect wave (rectangular wave)	off

To define the pulse period or the frequency of a wave, see the discussion <u>"Configuring the 2487 Detector"</u> in <u>Section 3.2.6, Operating Other Detector Functions</u>.

To program a threshold event:

- 1. Press the **METHOD** (Shift A/B) key on the 2487 Detector keypad. The Method choice list is displayed (Figure 3-20).
- Press 2, Threshold events. An active field (AU) for entering the threshold is displayed. When you begin to enter a number in the AU field, additional fields are displayed (Figure 3-22).

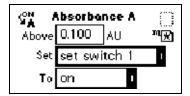


Figure 3-22 Threshold Events Screen

- 3. Press Enter to advance to the next (Set) field, or press the s and t key to move among the three fields on the threshold events screen.
- When the Set field is active, press Enter to display the threshold events choice list, or press the number corresponding to the event you are programming (<u>Table 3-5</u>).
- When the **To** field is active, press Enter to display the options in <u>Table 3-6</u>, or press the number corresponding to the threshold parameter you are programming (<u>Table 3-6</u>).
- 6. Press A/B to set the threshold on the other channel.

Storing a Method

A method consists of all programmable parameters on the absorbance and associated screens, as well as timed and threshold events. You can store the current method by selecting a location from 1 to 10. To store a method:

- 1. Press METHOD (Shift A/B) to return to the Method choice list (Figure 3-20).
- 2. Press 4, Store method *. A method number field appears (Figure 3-23).



Figure 3-23 Storing a Method, Method Number Field

Stop Attention: There is no warning message if the method number you select is already being used by a previously stored method. When you enter a number and press **Enter**, the current method conditions are stored, overwriting any previous method stored in the same slot.

3. Enter a number from 1 to 10 and press Enter. A brief message ("Storing * as method *n*") appears. When the display returns to the Method choice list, the method number you selected is displayed within the method icon. That method remains active until you retrieve another method or reset the detector to default conditions (Method *).

Retrieving a Method

To retrieve a previously stored method:

- 1. Return to the Method choice list by pressing METHOD (Shift A/B).
- 2. Press **3**, **Retrieve a method**. The last method number stored or retrieved is displayed in the method number slot box.
- 3. Enter the number of the method you wanr to retrieve and press Enter. A brief message ("Retrieving method *n*") appears.

When the display returns to the Method choice list, the method number you specified is displayed within the method number icon (<u>Table 3-1</u>).

Viewing Events Within a Method

To view timed and threshold events that make up a stored method:

- 1. Retrieve the method (see "Retrieving a Method"). Once you have entered the method number to retrieve, the Method choice list is displayed with the method number displayed within the method number icon.
- 2. Press 1 to view the Timed events or 2 to view Threshold events within the displayed method.

If you make a change to a timed or threshold event within a method, the asterisk is displayed (Method *) indicating that the method (*) is no longer the same as the stored method you retrieved in step 1. You can then store the method containing the altered event(s) in the same storage slot.

Resetting a Method

Resetting a stored method is a two-step process. First, you reset the current conditions to the defaults; then you save the defaults in one of the storage locations. <u>Table 3-3</u> lists the parameter default settings.

To clear one or more methods:

- 1. Return to the Method choice list by pressing METHOD (Shift A/B).
- 2. Press 5, Reset method *.

A message screen appears (Figure 3-24) asking you if it is okay to set current conditions to factory defaults.

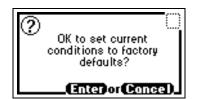


Figure 3-24 Reset Method Message

- 3. If you press Enter:
 - All timed events are deleted.
 - All threshold events are disabled.
 - All other operating parameters of the method (λ , AUFS, etc.) are set to defaults.

If you press Cancel (Shift 0), the display returns to the Method choice list.

Note: To prevent loss of the current conditions before you clear the method, store them in one of the available storage slots. When you have cleared the storage slots, you can restore the previous conditions.

4. Press 4, Store method, and enter a storage location number.

To clear other stored methods, repeat step 4.

5. When you return to the Absorbance screen by pressing **HOME**, the method number icon displays an asterisk.

Clearing Events

You may want to clear only timed or threshold events without resetting any other operating parameters. To clear all active timed or threshold events:

- 1. Return to the Method choice list by pressing METHOD (Shift A/B).
- 2. Press 6, Clear events.

A message screen appears (Figure 3-25) asking you if it is okay to clear all active events.



Figure 3-25 Clear Events Message

- 3. If you press Enter:
 - · All timed and threshold events in the method are cleared
 - All other operating parameters of the method (λ , AUFS, etc.) are unaffected If you press Cancel (Shift 0), the display returns to the Method choice list.
- 4. When you return to the Absorbance screen by pressing **HOME**, the method number icon displays an asterisk.

3.4 Scanning Spectra

Overview

The 2487 Detector must make two types of scan to produce an absorbance spectrum:

- Zero scan A reference scan that characterizes the absorbance spectrum of the solvent in the cuvette or the flow cell.
- **Sample scan** An absorbance scan of the analyte in solvent (after subtracting out the zero scan of the solvent) to provide the actual spectrum of the sample.

The 2487 Detector can measure the spectrum of a sample using either the cuvette or the flow cell. See <u>Section 3.4.7, Scanning Using the Cuvette</u>, and <u>Section 3.4.8, Scanning</u> <u>Using a Static Flow Cell</u>, for scanning procedures.

Note: When using the cuvette, if the contents of the flow cell change, you must rerun the zero scan.

Before You Begin

Before you run a spectrum scan, you need to specify the following parameters:

- λ **1** Starting wavelength. Scanning begins at this wavelength.
- $\lambda 2$ Ending wavelength. Scanning ends at this wavelength.
- **Pace** Rate of scanning in nm/min. Determines how fast the scan is output and data is acquired. The scan data is acquired at the highest possible resolution for the specified pace. When the pace is set to a very high number, the resolution is reduced, as in the following examples and Figure 3-26.

 Table 3-7 Pace and Sampling Resolution Examples

Pace (in nm/min)	Sampling Resolution (in nm)	
100 and less	0.5	
200	1.0	
400	2.0	

Figure 3-26 shows two scans of anthracene overlaid. At a pace of 1000 nm/min, the overlaid scan (with open circles) shows a reduced number of points scanned, lowering the resolution in comparison with the original scan done at a pace of 100 nm/min.

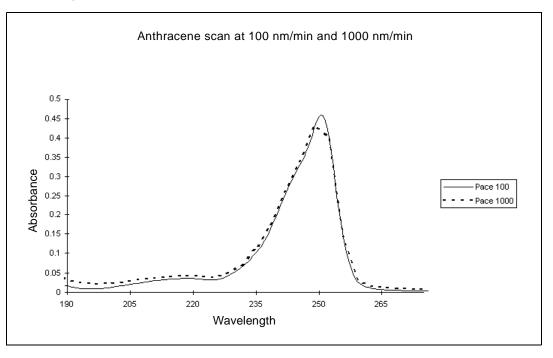


Figure 3-26 Scan of Anthracene at 100 nm/min and 1000 nm/min

Note: The higher the number you enter in the Pace field, the lower the resolution of the scan.

 Tickmarks – This value allows tickmarks to be generated at the specified wavelength increment to assist in the interpretation of charted data.

<u>Figure 3-27</u> shows a 190 to 600 nm scan of erbium standard in a cuvette at a pace of 200 nm/min, with tickmarks specified every 20 nm. <u>Figure 3-28</u> shows the same scan without tickmarks.

• AUFS – The sensitivity setting for scaling the charted spectrum.

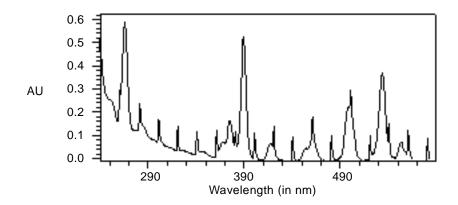


Figure 3-27 Scan of Erbium Standard in a Cuvette from 190 nm to 600 nm at a Pace of 200 nm/min with Tickmarks Every 20 nm

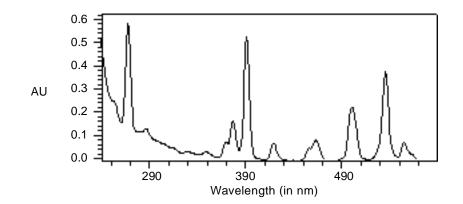


Figure 3-28 Scan of Erbium Standard in a Cuvette from 190 nm to 600 nm at a Pace of 200 nm/min Without Tickmarks

You enter these parameters when you select the type of scan, either zero or sample.

When you select a zero scan:

- The detector displays three additional screens, labeled 2 of 4, 3 of 4, and so on (Figure 3-30).
- You can change all parameters on these screens, including starting and ending wavelength, and the pace parameter.

When you select a sample scan:

• The detector displays two additional screens labeled 2 of 3 and 3 of 3 (Figure 3-30).

• You cannot change the starting and ending wavelength parameters or the pace parameter.

When you run the zero scan, you set up the starting and ending wavelengths, pace, tickmarks, and sensitivity for both the zero scan and subsequent sample scans. You should try to run sample scans within 15 minutes of the baseline zero scan.

The most recent zero scan performed or retrieved remains current until you perform or retrieve another scan. The zero scan should be appropriate for the subsequent sample scans you perform. The sample scan uses the starting and ending wavelengths and pace from the most recent zero scan. Only when these parameters are identical for both the zero and sample scans can the zero scan be subtracted.

You use the **SCAN** key (**Shift Chart Mark**) to run a new zero or sample scan, store, review, subtract and review, and replay stored or existing scans.

During a sample scan, data is charted out the 2487 Detector analog Channel A using the specified AUFS setting. At the same time, sample energy at 150 nA/V is charted out over Channel B.

During a zero scan, the data is charted out the 2487 Detector analog Channel A. At the same time, reference energy at 150 nA/V is charted out Channel A at the AU specified on Channel A.

3.4.1 Scanning New Spectra

To specify a new spectrum:

1. Press SCAN (Shift Chart Mark). A Scan choice list is displayed (Figure 3-29).



Figure 3-29 Scan Choice List

- Press 1, New scan, or use the s and t keys to move through the Scan choice list. The detector displays the first of three parameter screens for a Sample scan or four parameter screens for a Zero scan (Figure 3-30).
- 3. Press Next to advance through the New scan parameter screens.
- 4. On the first New scan screen, specify the type of scan:

- Press 1 for sample scan, or press Enter to display the choice list. The detector displays two additional screens.
- Press 2 for zero scan, or press Enter to display the choice list. The detector displays three additional screens.

All parameters are displayed on the first New scan screen for both a zero and a sample scan. You can return to screen 1 to review the parameters for either scan type by pressing **Next** from the Run screen (screen 3 of 3 for sample scan or 4 of 4 for zero scan).

Note: You can press Run from any New scan screen.

<u>Table 3-8</u> provides the defaults and ranges for all parameters for both zero and sample scans.

Parameter	Screen	Scan Type	Units	Range or Default
Туре	1	Sample and zero	n/a	Sample scan: 1 Zero scan: 2 Default: 1
λ range	2	Zero scan only	nm	Range: 190 to 700 nm Default: 190 to 700 nm
Pace	2	Zero scan only	nm/min	Range: 30 to 1000 nm/min Default: 100 nm/min
AUFS	2 or 3	Sample and zero	AU	Range: 0.0001 to 4.0000 Default: Last number entered
Tickmark (Mark each nm)	2 or 3	Sample and zero	nm	Range: 10 to 100 Default: Last number entered

Table 3-8 Sample and Zero Scan Parameters

Zero Scan

To program a zero scan:

- 1. Press SCAN, then 1, New scan, and press 2, Zero scan.
- 2. Press Next to advance to the second Zero Scan parameter screen.
- 3. Enter the starting wavelength for the zero scan, then press Enter.
- 4. Enter the ending wavelength for the zero scan, then press Enter.
- 5. Enter a value in the Pace field for the rate at which the 2487 Detector scans the specified wavelength range. The default is 100 nm/min. The allowable range is from 30 to 1000 nm. Refer to Figure 3-26, which shows two scans of anthracene overlaid, one at 100 nm/min and one at 1000 nm/min.

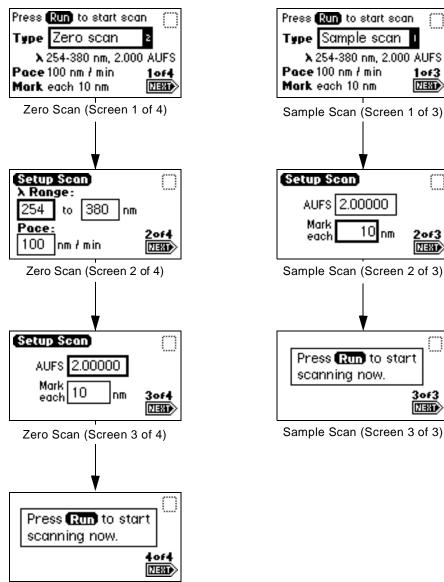
Note: The higher the number you enter in the Pace field, the lower the resolution of the scan.

- 6. Press Next to advance to the third Zero Scan parameter screen.
- 7. Enter the AUFS value, then press Enter.
- 8. To specify tickmarks, enter a number from 10 nm to 100 nm, then press Enter.
- 9. Press CE to disable tickmarks (changes the value to OFF).

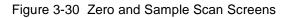
Refer to Figure 3-27 and Figure 3-28 for examples of scans made with tickmarks on or off.

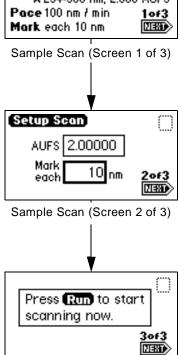
10. Press Run to start the zero scan, or press Next to return to the first zero scan parameter screen to check your parameters, then press Run.

When the 2487 Detector completes running the zero scan, it returns to the Scan choice list.



Zero Scan (Screen 4 of 4)





Sample Scan (Screen 3 of 3)

Sample Scan

Note: Run the zero scan before running the sample scan. To ensure identical flow cell and solvent conditions, you should run the sample scan for the corresponding zero scan within 15 minutes of running the zero scan.

To run a sample scan:

- 1. Set up the zero (or reference) scan by following the steps in the zero scan procedure given earlier.
- 2. When you are ready to run the sample scan, return to the first New scan screen and press 1, Sample Scan.

The parameters for wavelength range, AUFS, Pace, and Mark (tickmark) you entered for the corresponding zero scan are displayed.

- 3. Press **Next** to advance to the second sample scan screen. If desired, you may change the entries in the AUFS and Mark each fields.
- 4. Press Next to advance to the third sample scan screen, then press Run to run the sample scan.

To return to the Scan choice list at the completion of the sample scan, press SCAN (Shift Chart Mark).

Running the Scan

After you press **Run**, a brief message ("Initializing") is displayed. The Scanning screen shows the progress of the scan in nanometers across a progress bar (Figure 3-31).

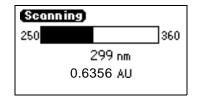
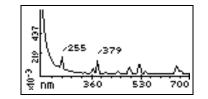


Figure 3-31 Scanning Progress Bar

For a zero scan, instantaneous energy in nanoAmps (nA) is displayed on the Scanning screen.

For a sample scan, absorbance in absorbance units (AU) is displayed.



After a brief pause, the detector displays a sample scan graphically (Figure 3-32).

Figure 3-32 Sample Erbium Scan Graphical Display

 Press Next to display up to four highest peaks scanned (if four are found) within the specified range. <u>Figure 3-33</u> shows the display of the four highest peaks for the sample erbium scan shown in <u>Figure 3-32</u>.

[λ Range	: 190-700	nm
nm	AU	
1 255	0.2228	
2 379	0.1931	
≥ 523	0.1460	
4 652	0.1245	NEXT>

Figure 3-33 Sample Erbium Scan Four Highest Peaks

- 6. Press **Next** to return to the graphical display.
- 7. Press Scale (Shift TRACE) to change the scale (zoom in on one part) of the display. You can alter the following four scaling parameters:
 - $\lambda 1$ minimum wavelength displayed.
 - $\lambda 2$ maximum wavelength displayed.
 - AU1 minimum absorbance displayed. The default is auto.
 - AU2 maximum absorbance displayed. The default is auto.

This function allows you to zoom in on various sections (artifacts) of the spectrum. The scale of the spectrum is affected by the AUFS setting.

 Press Next to advance through the four scaling parameters. Figure 3-34 shows the sample scanned in Figure 3-32 scaled by changing the wavelength parameters to 225 and 600 nm.

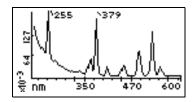


Figure 3-34 Sample Erbium Scan with λ 1 Changed to 225 nm and λ 2 Changed to 600 nm

- 9. Once you have changed one or more scaling parameters, press Enter to reformat the graphical display.
- Once the scan is redisplayed, press Next to display the four highest peaks of the scaled scan (Figure 3-35).

λ Range: 220-600 nm)			
	nm	AU	
- 1	255	0.2228	
2	379	0.1931	
Э	523	0.1460	
4	241	0.0949	DEST

Figure 3-35 Four Highest Peaks from the Scaled Sample Erbium Scan

11. Press Next again to return to the sample scan display.

<u>Figure 3-36</u> shows a series of scans of anthracene dissolved in acetonitrile to display the use of the Scale function. The zero scan is not displayed.

For the scaling parameters AU1 and AU2, the default is **auto**. You can change the AU parameter based on the absorbance of the spectrum. To return the default to **auto**, press **CE**.

 When you have finished manipulating the sample scan graphical display, press SCAN (Shift Chart Mark) to return to the Scan choice list. To store the scan, see <u>Section 3.4.2, Storing a Spectrum</u>.

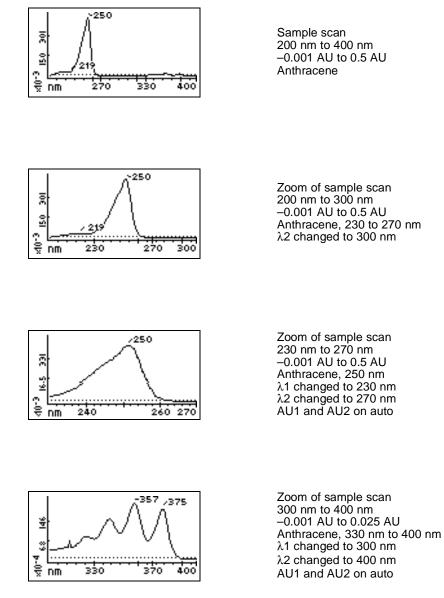


Figure 3-36 Series of Scans of Anthracene in Acetonitrile

3.4.2 Storing a Spectrum

Once you have run a spectrum, you can store it for later review, subtraction, or playback. You may store up to five spectra. To store a spectrum:

- 1. From the graphic display of a sample scan, return to the first Scan screen by pressing SCAN (Shift Chart Mark).
- 2. Press 2, Store last scan. A slot number box appears (Figure 3-37).

Note: When you select **Store last scan**, you are storing the zero scan and the sample scan as a pair.

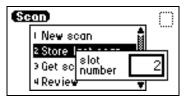


Figure 3-37 Storage Slot Number Box

- 3. Enter a number from 1 to 5.
- 4. Press Enter to store the last sample scan, paired with its zero scan.

3.4.3 Getting Information About a Stored Spectrum

You can obtain information about a stored spectrum as follows:

- 1. Press SCAN (Shift Chart Mark) to view the Scan choice list.
- Press 3, Get scan info. A slot number box appears with a default of "Last" (for the most recently stored spectrum).
- 3. Press Enter to get information about the last stored spectrum, or type the number (1 to 5) of the stored spectrum for which you want information, then press Enter.

A screen containing the following information appears:

- Storage slot number of the selected scan (or "Last scan")
- λ range Displays the wavelength range for the selected spectrum
- · Pace Displays the pace for the selected spectrum
- 4. Press Enter to dismiss the information screen, and return to the Scan choice list.

Once you have stored a spectrum, you can retrieve it for review from one of the five available storage slots by selecting the Review option from the Scan choice list. To review a spectrum:

- 1. Press SCAN (Shift Chart Mark) to view the Scan choice list.
- 2. Press 4, Review.

Note: When you select **Review**, you actually are retrieving the zero scan and the sample scan which were stored as a pair.

- 3. Enter the storage slot number (1 to 5) of the spectrum you want to review.
- 4. Press Enter. The message "Retrieving spectrum n" is displayed.

After a brief wait, the stored spectrum is retrieved and displayed.

Once you retrieve a spectrum for review, you can view it graphically and adjust the wavelength and AU ranges, if necessary. You can also run a new sample scan based on the retrieved zero scan.

3.4.5 Subtracting a Spectrum

Once you have stored more than one spectrum, you can create a difference spectrum.

Note: The current spectrum is the spectrum being subtracted from; the stored spectrum whose slot number you enter is the spectrum being subtracted out.

To subtract and review the difference spectrum:

- 1. Press SCAN (Shift Chart Mark).
- 2. Press 5, Subtract & review.

Note: In order to subtract one spectrum from another, the starting and ending wavelengths (λ 1 and λ 2) and the pace of both spectra must be identical.

- 3. Enter the storage slot number (1 to 5) of the spectrum you want to subtract out from the current (or retrieved) spectrum.
- 4. Press Enter. The message "Subtracting spectrum n" is displayed.

The detector reviews and subtracts the spectrum specified from the current spectrum and, after a brief wait, displays the difference spectrum.

Once the difference spectrum is displayed, you can store the results in one of the five storage slots.

3.4.6 Replaying a Spectrum

You can play back the current spectrum or a stored spectrum in real time using the Real-time replay function on the Scan choice list. The 2487 Detector plays back the selected spectrum in real time both on the detector display and out the analog connector to the chart or A/D device in a data collection system. Once you retrieve a spectrum for playback, the 2487 Detector displays it graphically, and you can adjust the AUFS. Sample energy is not charted during a replay.

Note: If you adjust the AUFS on a spectrum for replay, the adjusted spectrum displays only on the charted output, not on the 2487 Detector graphical display.

To replay a spectrum:

- 1. Press SCAN (Shift Chart Mark).
- 2. Press 6, Real-time replay.
- 3. Enter the storage slot number (1 to 5) of the spectrum you want to play back. The default is the last spectrum acquired.
- Press Enter. After a pause to retrieve the selected spectrum, the detector begins to play back the spectrum on the analog connection. Then the spectrum is displayed graphically.

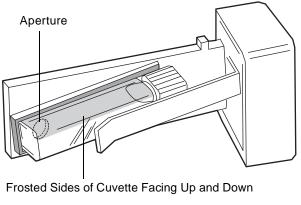
3.4.7 Scanning Using the Cuvette

The 2487 Detector cuvette option allows the operator ease of use in:

- · Sample handling
- · Instrument verification and qualification

The 2487 Detector cuvette option is useful in comparing the spectra of different batches of mobile phase before you use it in HPLC applications.

The 2487 Detector uses a standard 10 mm path length spectrophotometric cell (quartz cuvette). You insert the cuvette with the frosted sides facing up and down in the cuvette holder (Figure 3-38) and place it in the detector flow cell assembly.



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Note: Because the scan is actually a composite of both the contents of the cuvette and the flow cell, you need to perform cuvette scans under identical flow cell conditions. If you store spectra and acquire new spectra for subtraction, you need to be aware of differences, if any, in flow cell conditions.

Ideally, you should perform both the zero and sample scans using the cuvette when the HPLC instruments are in the idle or static state, under identical flow cell conditions.

Attention: Handle the cuvette gently and on the frosted sides only. Fingerprints on the clear quartz interfere with the light path and compromise the integrity of cuvette scanning operations.

Before You Begin

Note: To ensure accurate results, be sure to use a 10 mm path length quartz cuvette and to use matched pairs (from the same manufacturing lot) of quartz cuvettes for your zero and sample scans.

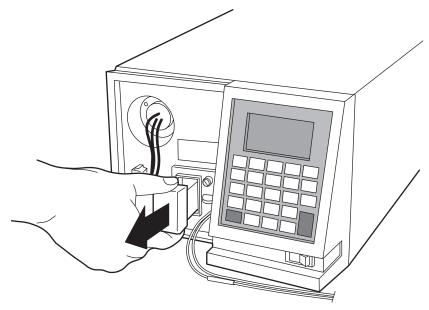
Before beginning a scan using the cuvette:

- 1. Flush the flow cell with the eluent you are going to scan.
- 2. To ensure a clear and clean cuvette, wipe the clear portion of the cuvette with low lint, nonabrasive tissue wipes.

Cuvette Scanning Procedure

To begin a cuvette scan:

- 1. Remove the 2487 Detector left front panel cover.
- 2. To remove the cuvette holder, slide it toward you (Figure 3-39).



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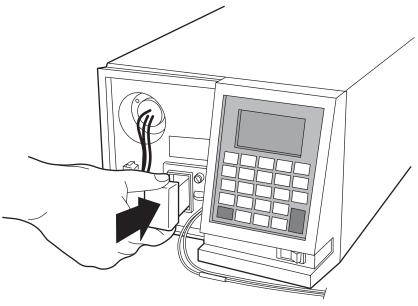
Figure 3-39 Removing the Cuvette Holder

3. With the spring guide facing you, gently insert the cuvette (containing eluent) up under the guide, with the cap facing upward (into the holder) and the frosted sides of the cuvette facing up and down. Refer to Figure 3-38.

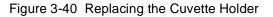
Note: Ensure that you have enough liquid (3 mL) in the cuvette so that when it is inserted into the holder, you can see liquid through the cuvette holder aperture, that is, the liquid completely covers the aperture.

Note: Since the cuvette holder is angled, use your thumb or forefinger to ensure the cuvette is secure in the slot and does not slide forward. Ensure that it does not become dislodged when you are replacing the cuvette holder.

 Gently guide the cuvette holder back into the flow cell assembly until it sits in position securely (<u>Figure 3-40</u>).



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5. Reinstall the detector left front panel cover.



Attention: To prevent invalid subsequent chromatographic results, be sure to remove the cuvette from the 2487 Detector and replace the empty holder after running your cuvette scans.



Attention: To maintain optimum system performance, be sure to replace the left front panel cover before resuming normal operation of the 2487 Detector.

- 6. Insert a reference cuvette containing the mobile phase standard and run a zero scan.
- 7. Replace the reference cuvette with a cuvette containing your analyte dissolved in mobile phase solvent and run a sample scan.

8. Use the storage, review, subtract and review, and replay functions to analyze the data obtained.

3.4.8 Scanning Using a Static Flow Cell

Note: Before you scan using a static flow cell, be sure to remove the cuvette and replace the empty cuvette holder.

To run a spectrum using a static flow cell:

- 1. With a syringe, fill the flow cell with the mobile phase or solvent in which your sample is dissolved.
- 2. Run a zero scan following the procedure in Section 3.4.1, Scanning New Spectra.
- 3. With a syringe, fill the flow cell with the analyte, then run a sample scan following the procedure in <u>Section 3.4.1, Scanning New Spectra</u>.

Use the storage, review, subtract and review, and replay functions of the 2487 Detector to compare the scanned data.

3.4.9 Conserving Lamp Life

To conserve the lamp without shutting down the 2487 Detector, you can leave the instrument on and turn off only the deuterium lamp.

Note: If the 2487 Detector is operating under remote control, you can program the controller to turn the lamp off and on without using the detector front panel.

Note: We recommend that you program the lamp to shut off or turn the lamp off manually only if the "lamp off time" is more than 4 hours.

Without powering down the system, you can conserve lamp life by:

- Turning power to the lamp off and on again manually
- Programming a timed event to turn the lamp off and on again
- Programming the lamp to turn off and on again using the external contact closure

To turn power to the lamp on and off manually, use the Lamp keypad function (Shift 1). When the lamp is turned off, the Absorbance screen displays the words "Lamp off" and the lamp icon appears with an X through it.

Use the Lamp key (Shift 1) to:

- Shut the lamp off or turn the lamp on manually
- Display the number of times the lamp has ignited

- Display the hours and minutes the lamp has been turned on:
 - During the current run
 - Since installation

To turn off the lamp manually from the detector front panel:

1. Press Lamp (Shift 1) from the keypad. The lamp control screen is displayed (Figure 3-41).

Press (Lamp) again to turn lamp (1997	®
Lamp has been on 0 h 07 m Total since installed 12 h 03 m	Times fired 6

Figure 3-41 Lamp Control Screen

The lamp control screen shows:

- The amount of time, in hours and minutes, the lamp has been on since the most recent startup
- The total time the lamp has been on since installation
- The number of times the lamp has been fired up
- Press Lamp (Shift 1) again to turn the lamp off. The Absorbance screen is displayed, with an X through the lamp indicator icon, and the display shows the words "Lamp off." See <u>Figure 3-42</u>.

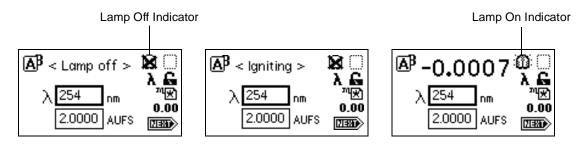


Figure 3-42 Lamp Off/On Sequence

To turn on the lamp manually:

- When there is an X through the lamp icon on the Absorbance screen, press Lamp (Shift 1). The lamp control screen is displayed again with 0 hours and 00 minutes in the "Lamp has been on" field.
- Press Lamp (Shift 1) again to turn the lamp on. The Absorbance screen is displayed with the message "Igniting" (Figure 3-42). The lamp may take up to one minute to illuminate. Once the lamp is illuminated, the display returns to the Absorbance screen with the X removed from the lamp icon.

You can conserve lamp life by programming it to turn on and off automatically, for example, overnight, using a timed event method.

To program the lamp to turn on or off, you can select the **Timed events** option in the Method choice list, or program the lamp through one of the external contact closures.

- See <u>Section 3.3.6, Programming Timed Events, Threshold Events, and Methods</u>, and <u>Table 3-4</u> for more information on programming the lamp to turn on or off using a timed event.
- See the discussion <u>"Configuring Event Inputs (Contact Closures)</u>" in <u>Section 3.2.6</u>, <u>Operating Other Detector Functions</u>, for more information on programming the lamp through the external contact closure.

3.4.10 Shutting Down the 2487 Detector

Before you power off the 2487 Detector, you need to remove any buffered mobile phase present in the fluid path.



Attention: To avoid damage to your column, remove the column before you perform the following procedure.

Removing Buffered Mobile Phase

To remove mobile phase from the fluid path of the 2487 Detector:

- 1. Replace the buffered mobile phase with 100% HPLC-quality water and flush the system for 10 minutes at 3 mL/min.
- 2. Replace the 100% water mobile phase with a solution of 90% methanol: 10% water, and flush the system for 10 minutes at 3 mL/min.

Follow the recommended procedures for injector purging and priming for the pump in use with your HPLC.

Shutting Down the 2487 Detector

To shut down the detector, press the **On/Off** switch located on the front lower-right corner of the unit.

4 Maintenance Procedures

4.1 Introduction

Read the appropriate maintenance instructions before starting the procedures for any of the tasks in this chapter. If you are uncertain about how to perform the procedures, call the Waters[®] Technical Service Department to have a trained service representative perform the procedure.

Waters Technical Service

For problems with HPLC equipment, computer software or hardware other than for the Waters 2487 Dual λ Absorbance Detector, consult the documentation for the applicable instrument or program.

If you encounter a problem with the 2487 Detector that you cannot troubleshoot, contact Waters Technical Service at 1-800-252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

Spare Parts

Stock the recommended spare parts to minimize downtime. Refer to <u>Appendix B, Spare</u> <u>Parts</u>, for a list of recommended spare parts. Appendix B lists only those parts replaceable by the user. Parts not included in the list require replacement by a trained service representative.

Safety Precautions

When you perform the maintenance procedures in this chapter on your 2487 Detector, keep the following safety considerations in mind:



Caution: To prevent the possibility of electrical shock, never disconnect an electrical assembly (including all interface cables) while power is applied to the 2487 Detector.



Caution: When you handle eluents, change tubing, or operate the 2487 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the eluents. Refer to the Material Safety Data Sheets for the eluents in use.



Caution: To avoid the possibility of electrical shock, always turn power to the 2487 Detector off and disconnect the power cord before performing maintenance procedures.

Removing the Left Front Panel Cover



Attention: Since there are no user-serviceable parts inside the 2487 Detector, the top cover should not be removed. However, some procedures require that you remove the left front panel cover which contains the Waters 2487 Dual λ Absorbance Detector nameplate.

Note: You can use the left front panel cover to hold small parts when removing and replacing the 2487 Detector flow cell.

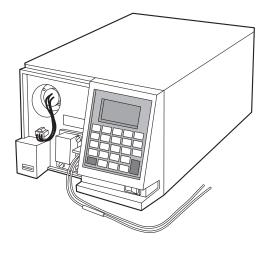


Attention: To maintain optimum system performance, be sure to replace the left front panel cover before resuming normal operation of the 2487 Detector.

To remove the left panel front cover:

- 1. Holding onto the top of the cover, gently pull the bottom away from the unit.
- 2. Gently remove the top of the cover and store nearby.

Figure 4-1 illustrates the 2487 Detector with the left front panel cover removed.



TP01498

Figure 4-1 View of the Waters 2487 Dual λ Absorbance Detector with the Left Front Panel Cover Removed

4.2 Routine Maintenance

The Waters 2487 Dual λ Absorbance Detector requires minimal routine maintenance. To achieve optimal performance:

- 1. Replace solvent reservoir filters in your HPLC system regularly.
- 2. Filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.
- 3. Flush buffered mobile phases out of the detector with HPLC-grade water followed by a 5 to 10 percent methanol solution each time the detector is shut down. This process prevents:
 - Plugging of the solvent lines and flow cell
 - · Damage to the components
 - · Microbial growth

4.3 Inspecting, Cleaning, and Replacing Parts of the Flow Cell

A dirty flow cell can cause baseline noise, decreased sample energy levels, calibration failure, and other problems with operation of the 2487 Detector.

There are two phases in cleaning the flow cell:

- Flushing
- Removing and cleaning

If flushing is ineffective, remove and clean the flow cell. Replace flow cell components as required.



Attention: Always replace the flow cell gaskets when cleaning, rebuilding, or replacing other flow cell components.

This section provides information about the following procedures:

- Flushing the flow cell
- Removing and cleaning the flow cell
- Disassembling and reassembling the flow cell

4.3.1 Flushing the Flow Cell

Flush the flow cell after each shutdown, or when dirty. To flush the flow cell:

- 1. Discontinue the eluent flow, and remove the column.
- 2. Attach tubing from the 2487 Detector to the injector outlet (from which the column has been removed). Flush the mobile phase from the detector with a miscible solvent and water (unless the mobile phase is miscible with water).
- 3. Flush the detector with HPLC-quality water to remove contaminants from the flow path.
- 4. Pump 6 M nitric acid through the flow cell to clean the internal pathways.
- 5. Flush again with HPLC-quality water until the solvent is neutral.
- 6. Reattach the column to resume flow of the mobile phase. Use an intermediary solvent if you are using a mobile phase that is not miscible in water.

4.3.2 Removing and Cleaning the Flow Cell

If flushing the flow cell is not effective, follow the procedures to remove the flow cell and inspect for dirty or broken windows or dirty gaskets. Clean and replace parts if necessary.

Before removing the flow cell assembly, purge the flow cell with nitrogen and allow to dry as follows:

- 1. Connect the nitrogen supply line to the sample inlet. Route the sample line to waste.
- 2. Purge the cell for 25 to 30 minutes at 15 psi to 20 psi.
- 3. Allow the flow cell to dry completely.
- 4. Disconnect the 2487 Detector inlet/outlet tubing from the main column connection and cap.

4.3.3 Disassembling and Reassembling the Flow Cell

Before You Begin

Take the following precautions when disassembling and reassembling the flow cell:

- To prevent contamination, use powder-free finger cots or gloves when touching the flow cell lenses or window.
- Take care to prevent scratching of the flow cell parts.
- Use a clean nonparticulating cloth or a similar clean surface in the work area where you are removing, rebuilding, and replacing the flow cell.



Attention: Always replace the flow cell gaskets when cleaning, rebuilding, or replacing other flow cell components.

Tools Required

You need the following items to remove, clean, and replace the flow cell:

- 1/4-inch flat-blade screwdriver
- Torque wrench set to 128 in-oz (8 in-lb or 0.904 Nm) with 1/4-inch flat-blade screwdriver bit
- Plastic tweezers
- Powder-free finger cots or gloves
- Lint-free swab
- · Ethyl alcohol or methanol
- Nitrogen

Save all parts after removal unless otherwise indicated. Most of the removed parts are needed when you reinstall a flow cell.

Removing the Flow Cell Assembly

To remove the flow cell assembly:

- 1. Power down the 2487 Detector.
- 2. After flushing and drying the flow cell (see <u>Section 4.3.1, Flushing the Flow Cell</u>), disconnect and cap the inlet and outlet LC tubing to the detector.
- 3. Remove the left front panel cover.
- 4. Using a 1/4-inch flat-blade screwdriver, loosen the three captive screws on the flow cell assembly front plate (Figure 4-2).
- 5. Gently pull the assembly toward you (Figure 4-3).

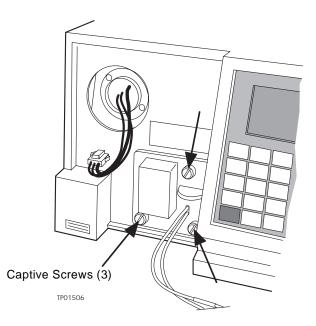


Figure 4-2 Unscrewing the Flow Cell Assembly Captive Screws



Figure 4-3 Removing the Flow Cell Assembly

6. Once the flow cell assembly is removed from the 2487 Detector chassis, remove the cuvette holder from the flow cell assembly (Figure 4-4).

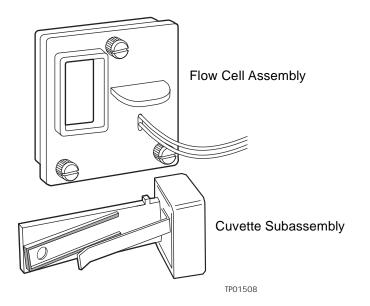


Figure 4-4 2487 Detector Flow Cell Assembly with the Cuvette Subassembly Removed

7. Place the flow cell assembly on a flat clean surface.

4.3.3.1 Disassembling the Flow Cell



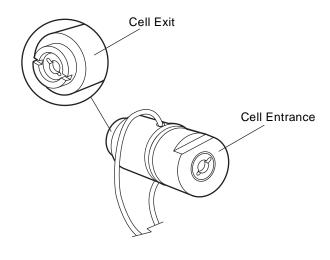
Attention: To prevent contamination, use powder-free finger cots or gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit[™] Flow Cell or when removing or replacing the flow cell within its assembly.

The Waters patented TaperSlit Flow Cell consists of:

- Flow cell body
- Cuvette lens
- Split ring (cuvette lens holder)
- · Cuvette lens screw
- Lens mount screw
- Exit window
- Exit window mount

- Entrance lens holder
- Entrance lens
- Entrance lens mount
- Two gaskets

Figure 4-5 shows the Waters TaperSlit Flow Cell, with frontal views of both ends.



TP01495

Figure 4-5 Waters TaperSlit Flow Cell

For replacement parts for the Waters TaperSlit Flow Cell, use the Flow Cell Rebuild Kit (P/N WAS081346).

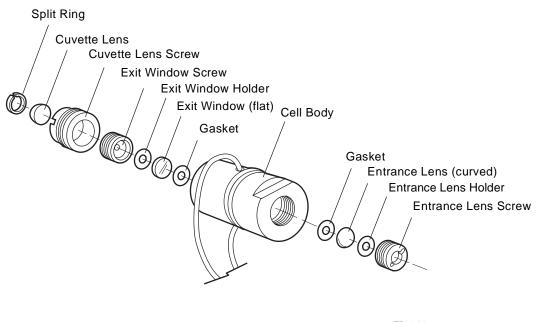
Note: Use nitrogen to clean the flow cell. Use ethanol or methanol to clean the lenses and window.

To remove the individual parts of the flow cell for cleaning or replacement:

- 1. With the notches on the flow cell cuvette lens end facing you, use the flat-blade screwdriver or a coin to remove the cuvette lens screw (Figure 4-6).
- 2. Insert the cotton end of the lint-free swab into the cuvette end of the flow cell assembly and remove the split ring and the cuvette lens.
- 3. Using the screwdriver, remove the exit lens mount screw.
- 4. Using plastic tweezers, pull out the exit window from inside the exit lens mount screw.

- 5. Using the plastic tweezers or the lint-free swab, remove the clear plastic gasket.
- 6. Flip the flow cell over to the entrance lens side.
- 7. Using the flat-blade screwdriver, remove the entrance lens screw.
- 8. Using the plastic tweezers, remove the entrance lens from inside the entrance lens screw.
- 9. Using the plastic tweezers or the lint-free swab, tap and remove the clear plastic gasket.

Figure 4-6 shows an exploded view of all parts of the flow cell within the flow cell assembly.



TP01520

Figure 4-6 Waters TaperSlit Flow Cell, Exploded View

4.3.3.2 Inspecting, Cleaning, and Replacing a Damaged Flow Cell Lens, Window, or Gasket



Attention: To prevent contamination, use powder-free finger cots or gloves when STOP disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit Flow Cell or when removing or replacing the flow cell within its assembly. Work on a clean flat surface, such as a nonparticulating cloth or similar surface.

To inspect or clean the parts of the flow cell (Figure 4-6) or to replace damaged parts of the flow cell such as the lenses, exit window, or gaskets, follow this procedure and the procedure in Section 4.3.3.3, Rebuilding the Flow Cell.

Note: Waters recommends that you replace the clear plastic gaskets each time you inspect and clean the flow cell.

- 1. Inspect each part of the flow cell removed in the procedure in <u>Section 4.3.3.1.</u> Disassembling the Flow Cell, for dirt.
- 2. Using ethanol or methanol, clean the contaminated part. Blow dry with nitrogen.
- 3. Using the Flow Cell Rebuild Kit (P/N WAS081346), replace any flow cell parts that are scratched, burred, damaged, or do not come clean using nitrogen.
- 4. Rebuild the flow cell following the procedure in <u>Section 4.3.3.3. Rebuilding the</u> Flow Cell.

4.3.3.3 Rebuilding the Flow Cell

After cleaning or replacing other flow cell parts, follow this procedure to rebuild the flow cell:

- 1. Using the plastic tweezers, remove the new clear plastic gaskets from the Flow Cell Rebuild Kit (P/N WAS081346), and inspect them for dirt.
- 2. Drop one clear plastic gasket into the groove at the bottom of the entrance lens end of the flow cell body.
- 3. Inspect the entrance lens and use nitrogen to blow dust off, if necessary.
- 4. Using plastic tweezers, drop the entrance lens into the flow cell body, curved side facing up.
- 5. With the tan-colored entrance lens holder curve facing down, use the torque wrench to tighten the entrance lens screw into the flow cell body to 128 in-oz (8 in-lb or 0.904 Nm).
- 6. Flip the flow cell body over to the exit window side.
- 7. Using plastic tweezers, inspect the second new gasket for cleanliness.

- 8. Drop the clear plastic gasket into the groove at the bottom of the cuvette lens end of the flow cell body.
- 9. Inspect the exit window for cleanliness. Using nitrogen, clean the exit window, if necessary.
- 10. Using plastic tweezers, place the exit window into the flow cell body.
- With the tan-colored exit window holder facing down, use the torque wrench to tighten the exit window screw into the flow cell body to 128 in-oz (8 in-lb or 0.904 Nm).

Note: To ensure complete compression of the gaskets, after replacing the exit window and torquing the exit window screw, and after replacing the cuvette lens and torquing the cuvette lens screw, you must flip the flow cell body and torque the entrance lens screw to 128 in-oz (8 in-lb or 0.904 Nm) a second time.

- 12. Flip the flow cell body again, and torque the entrance lens screw to 128 in-oz (8 in-lb or 0.904 Nm).
- 13. Flip the flow cell body again to the cuvette lens end, and torque the exit lens screw again to 128 in-oz (8 in-lb or 0.904 Nm).
- 14. With the cuvette lens curved side facing up, replace the cuvette lens in the cuvette lens screw mount.
- 15. Using your fingers (covered with powder-free finger cots or gloves), place the split ring onto the cuvette lens and press it firmly until it is flush on all sides.
- 16. Using the cuvette tool, screw the cuvette lens screw into the exit lens screw mount end of the flow cell body.
- 17. Torque the cuvette lens screw to 128 in-oz (8 in-lb or 0.904 Nm).
- 18. Follow the procedures in <u>Section 4.3.3.4</u>, <u>Replacing the Flow Cell</u>, to replace the flow cell into the flow cell assembly.

4.3.3.4 Replacing the Flow Cell



Attention: To prevent contamination, use powder-free finger cots or gloves when disassembling, inspecting, cleaning, or replacing parts within the Waters TaperSlit Flow Cell or when removing or replacing the flow cell within its assembly.

The 2487 Detector is shipped with a standard analytical flow cell installed. Replace the flow cell when:

- The flow cell becomes damaged
- You want to use one of the optional flow cells (<u>Table A-3</u>, Optional Waters TaperSlit Flow Cell Specifications)

Before you begin:

- 1. Unpack and inspect the new flow cell.
- 2. Power down the 2487 Detector.
- 3. Remove the left front panel cover.
- 4. Disconnect the 2487 Detector inlet/outlet tubing from the main column connection and cap.

To replace the flow cell:

- 1. Using a 1/4-inch flat-blade screwdriver, loosen the three captive screws on the flow cell assembly front plate (Figure 4-2).
- 2. Gently pull the assembly toward you.
- 3. Insert the new flow cell assembly into the 2487 Detector.
- 4. Tighten the 1/4-inch captive screws.
- 5. Reconnect the inlet/outlet tubing to your LC system.
- 6. Power up the detector.

4.4 Replacing the Lamp



Attention: Waters recommends that you change the 2487 Detector lamp when it repeatedly fails to ignite, or when the detector fails to calibrate. See <u>Table 5-1</u>, <u>Table 5-2</u>, and <u>Table 5-3</u>.

This section describes the procedure for removing and replacing the 2487 Detector deuterium lamp.

Lamp Characteristics

The intensity of the deuterium source lamp varies by wavelength as shown in Figure 4-7.

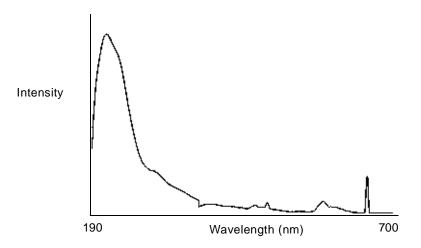


Figure 4-7 Deuterium Lamp Sample Beam Intensity Profile

Lamp Energy and Performance

As the lamps used in traditional absorbance detectors age, the signal-to-noise performance of the instrument degrades. Determining the longevity of "useful" lamp life can be difficult due to varying user requirements and individual lamp behavior.

The Waters 2487 Detector uses a combination of front-end electronics and lamp optimization software that make the best use of the energy present to optimize instrument performance. The design also compensates for the lamp energy changes that occur across the deuterium spectrum as well as lamp aging. This allows the 2487 Detector to operate at the same high signal-to-noise performance at longer wavelengths of the visible range, without the use of a second lamp, such as tungsten.

The 2487 Detector performs several self-diagnostic routines when it is powered up or when the **Calibrate** key is selected. One of these steps is the lamp optimization software routine. After the unit has verified the calibration of the monochromator, the instrument evaluates energy levels in a number of characteristic regions across the spectrum. The integration time of the front-end electronics is adjusted 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.

When operating the 2487 Detector, it is important to regularly invoke the lamp optimization software routine to maintain performance. The detector should be cycled off and on or the **Calibrate** key should be selected at least once a week.

Eventually, the lamp's signal becomes low enough that replacement is necessary. The energy value at which the 2487 lamp should be discarded is 15 at a wavelength of 225 nm. This corresponds to the cut-off used in the 2487 Detector's diagnostics. In practice, it is advisable that users set a higher value to avoid dropping below this value during an analysis.

Ultimately, the detector's performance is a function of the user's unique application. Noise and drift measurements are appropriate for evaluating performance and setting the boundaries for acceptable sensitivity limits. Waters guarantees 2000 hours of lamp life or one year of operation, which ever comes first. The detector's on-board diagnostics allow lamp usage to be recorded and the lamp serial number to be reported. You can replace lamps at any time that is appropriate to your method.

When to Replace the Lamp



Attention: Always run the Change Lamp diagnostic (see <u>Section 5.2.2</u>, <u>Using the</u> <u>Diagnostics</u>, the <u>"Using the Lamp, Display, and Keypad Diagnostics</u>" discussion) after you install a new lamp.

Replace the lamp on your 2487 Detector when:

- · It fails to ignite at startup
- When the energy level in the lamp causes a decrease in sensitivity to the point where the baseline is too noisy for your LC application

Always run the procedure in the discussion <u>"Recording Sample and Reference Beam</u> <u>Energies</u>" in <u>Section 3.3.2</u>, <u>Verifying the Detector</u>, each time you change the 2487 Detector lamp.

Performance requirements and permitted tolerances vary from application to application. If the lamp no longer provides an adequate signal-to-noise ratio for your specific application, replace it.

4.4.1 Removing the Lamp

Caution: To avoid eye damage from ultraviolet radiation exposure, turn off the unit and strictly adhere to the following procedures while changing the lamp.



Caution: Lamp housing becomes extremely hot during operation. To prevent burns to the skin, allow the lamp to cool for at least 30 to 60 minutes before removing.

To remove the lamp:

- 1. Turn off power to the 2487 Detector.
- 2. After turning off the unit, allow the lamp to cool for 30 to 60 minutes, minimum.
- 3. Remove the left front panel cover, as described in the discussion <u>"Removing the Left Front Panel Cover"</u> in <u>Section 4.1, Introduction</u>.
- 4. Remove power to the lamp (Figure 4-8).

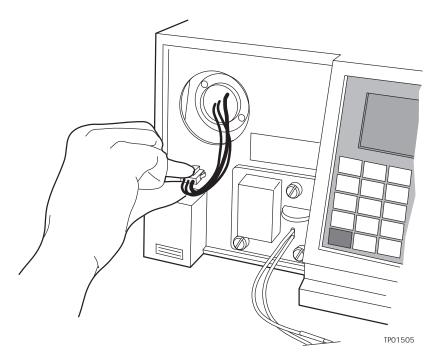


Figure 4-8 Lamp Assembly and Power Connector

- 5. Loosen the two captive screws in the lamp base (Figure 4-9).
- 6. Pull the lamp assembly out of the lamp housing.
- 7. Gently pull the lamp out (Figure 4-10).



Caution: Lamp gas is under slight negative pressure. To prevent shattering the glass, use care when disposing of the lamp.

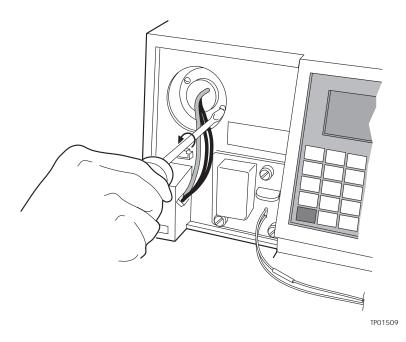


Figure 4-9 Loosening the Captive Screws at the Lamp Housing Base

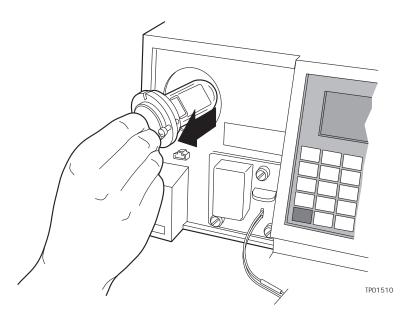


Figure 4-10 Removing the Lamp

4.4.2 Installing the New Lamp



Caution: To avoid the possibility of exposing your eyes to ultraviolet radiation, wear eye protection that filters ultraviolet light and keep the lamp in the housing during operation.

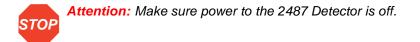


Attention: Do not touch the glass bulb on the new lamp. Dirt or fingerprints on the bulb affect detector operation. If the lamp needs cleaning, gently clean the bulb with ethanol and lens tissue. Do not use any abrasive tissue or apply excessive pressure.

Before you begin:

- 1. Unpack the lamp from its packing material. The new lamp may vary slightly from the lamp illustrated in <u>Figure 4-10</u>.
- 2. Inspect the new lamp and lamp housing.

3. Record the serial number located on a label attached to the lamp connector wire using the procedure in <u>Section 4.4.3</u>, <u>Recording the New Lamp Serial Number</u>.



To install the new lamp:

- Position the lamp so the cut-out located on the lamp base plate is at the 1 o'clock position (Figure 4-11), in line with the alignment pin on the lamp housing. There is no additional alignment required.
- 2. Gently push the lamp forward until it bottoms into position.
- 3. Tighten the two captive screws.
- 4. Reconnect the lamp power connector.
- 5. When ready to resume operation of the 2487 Detector, turn the power to the unit on. Allow at least 5 minutes for the lamp to warm up before resuming operations.



Attention: When you change the lamp, always power off the detector. After installing a new lamp, always power on the detector and wait at least five minutes for the new lamp to warm up.

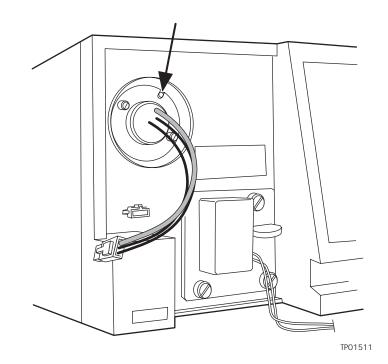


Figure 4-11 Aligning the Lamp

4.4.3 Recording the New Lamp Serial Number



Attention: Always run the Change Lamp diagnostic (see <u>Section 5.2.2, Using the</u> <u>Diagnostics</u>, the <u>"Using the Lamp, Display, and Keypad Diagnostics</u>" discussion) after you install a new lamp.



Attention: If you do not record the new lamp serial number following the procedure in this section, the lamp warranty is voided.

The 2487 Detector software allows you to record and store the serial number and date of installation of a new lamp so that you can periodically check its age and its number of ignitions.

To record the new lamp serial number:

1. Once the unit warms up, press the **DIAG** key.

- 2. Press 4, Lamp, display & keypad.
- 3. Press 1, Change lamp.

Note: Be sure to enter the 9-digit lamp serial number and not the lamp part number when performing this procedure.

4. Enter the 9-digit serial number of the new lamp in the active field (<u>Figure 4-12</u>). This field accepts numeric entries only.

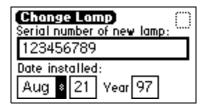


Figure 4-12 Change Lamp Screen

- 5. Press Enter to store the serial number and move to the Date installed field.
- 6. Select the month from the choice list.
- 7. Press Enter twice to update the month and move to the Day field.
- 8. Enter the number for the day of the month the lamp is installed, and press Enter to enter the date and move to the Year field.
- 9. Enter the year (last two digits only) and press Enter.
- 10. Press the HOME key. An "OK to store" message (Figure 4-13) appears.



Figure 4-13 Lamp Serial Number OK to Store Message

- 11. Press Enter to enter the serial number and date of installation of the new lamp or press Cancel to cancel the entry.
- 12. Once you press **Enter**, a confirmation message (<u>Figure 4-14</u>) appears. Press Enter to continue.
- 13. Perform a manual wavelength calibration (see <u>Section 3.3.3, Wavelength</u> <u>Calibration</u>).



Figure 4-14 Lamp Serial Number Confirmation Message

Attention: To run the verification procedures with the new lamp installed, recalibrate the detector or recycle power to the 2487 Detector after replacing the lamp.

4.5 Replacing Fuses

The fuse holder is located on the rear panel of the detector (Figure 4-15). Two fuses are shipped in place with the 2487 Detector, rated as indicated in <u>Section 2.2.2, Power</u> <u>Requirements</u>.

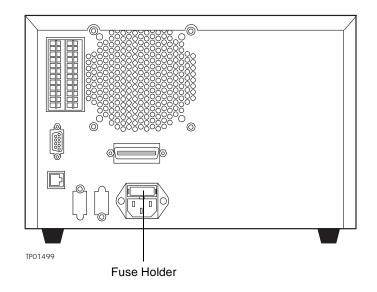
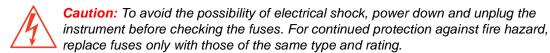


Figure 4-15 2487 Detector Rear Panel Fuse Holder



Identifying Faulty Fuses

Suspect a faulty fuse if:

- The 2487 Detector fails to power up.
- The display is blank.
- The fan does not turn on.

Replacing the Rear Panel Fuses

If you suspect the fuse is blown or faulty, replace it as follows:

- 1. Power off the 2487 Detector.
- Insert your fingers into the fuse holder slots on the rear panel of the detector. Exerting a minimum pressure, pull on the spring-loaded fuse holder and remove it from the rear panel (Figure 4-16).
- 3. Remove and discard the old fuse.
- 4. Make sure that the new fuse is properly rated for your requirements.
- 5. Insert the new fuse into the fuse holder.
- 6. Insert the fuse holder into the receptacle and gently push until it locks into position on the detector rear panel.

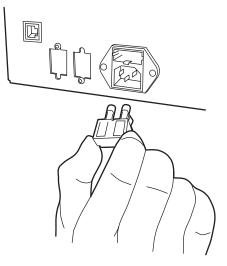


Figure 4-16 Removing and Replacing the Rear Panel Fuses and Fuse Holder

5 Error Messages, Diagnostics, and Troubleshooting

The Waters[®] 2487 Dual λ Absorbance Detector provides both user and service diagnostics to troubleshoot system problems.

- Error messages Describe powerup, calibration, and other error messages and recommended actions for correcting the errors.
- **Diagnostics** Describe user diagnostics for troubleshooting and configuring the detector.

5.1 Error Messages

5.1.1 Startup Error Messages

Startup diagnostics are executed automatically when power to the 2487 Detector is turned on. These diagnostics verify the proper operation of the detector electronics.

If one or more of the internal startup diagnostics checks fails, the detector:

- Beeps
- Displays an error message
- For serious errors, it displays the word "Error" in brackets (<Error>) in place of the run-time absorbance on the Absorbance screen.

Note: To prevent errors at startup, be sure the cuvette holder is empty, the flow cell contains degassed transparent solvent (methanol or water), and the left front panel cover is attached securely.

The tables in this section are organized as follows:

- Messages requiring you to perform corrective action. These include messages encountered at startup, and during calibration or operation.
- Messages requiring you to recycle power, then contact Waters Technical Service personnel if the error persists (see <u>"Waters Technical Service</u>" in <u>Section 4.1</u>). Most of these errors are encountered on startup.

<u>Table 5-1</u> provides startup, calibration, and operating error messages, descriptions, and recommended actions you can take to correct the problem.

Refer to <u>Section 1.2.2</u>, <u>Wavelength Verification and Test</u>, <u>Section 3.3.2</u>, <u>Verifying the</u> <u>Detector</u>, and <u>Section 3.3.3</u>, <u>Wavelength Calibration</u>, for verification and calibration information and procedures.

Error Message	Description	Corrective Action
Wavelengths span 370 nm: Order filter not in use	In dual wavelength mode: • If both selected wavelengths > 370 nm, the detector applies the second order filter to block unwanted UV light. • If both selected wavelengths < 370 nm, the detector removes the second order filter. • If the selected wavelengths bracket the 370 nm threshold, the detector does not apply the second order filter and issues a warning message that any data collected for wavelengths above 370 nm may contain inaccuracies because of possible UV light interference (second order effects).	Select wavelengths that are both above or both below 370 nm.
Lamp failure	Lamp indicates Off when it should be On.	Check lamp icon. Recycle power to the 2487 Detector. Replace lamp.

Table 5-1 Startup, Calibration, and Operating Error Messages

Error Message	Description	Corrective Action
Lamp lighting failure	The lamp failed to ignite.	Recycle power to the 2487 Detector. Check lamp power connection. Replace lamp.
Lamp external input conflict	A timed event or action from the unit's front panel attempts to change the lamp state in conflict with enabled lamp input contact closure.	Check contact closure status. Check timed events. Recycle power to the 2487 Detector.
Calibration not found	Stored calibration data not valid.	Perform manual calibration procedure.
Peak not found: Erbium <i>n</i> nm	Erbium filter calibration range does not contain a local maximum.	Remove cuvette. Is left front panel door closed? Flush flow cell.
Peak not found: 656 nm Deuterium	Unit sensors cannot determine 656 nm peak.	Remove cuvette. Is left front panel door closed? Flush flow cell.
Calibration unsuccessful: Peak out of range <i>n.nn</i> nm	Results of a calibration operation differ by more than 1.0 nm. Unit uses previously stored calibration points.	Remove cuvette. Is left front panel door closed? Flush flow cell.

Table 5-1 Startup, Calibration, and Operating Error Messages (Continued)

Error Message	Description	Corrective Action
Calibration differs: <i>n</i> nm	At startup, the unit performs a complete verification which includes remeasurement of all calibration points. New calibration points are compared to stored information from the most recent manual calibration. If any one of the points differs by more than 1.0 nm, the detector displays this message.	Recycle power to the 2487 Detector. Perform manual calibration. Contact Waters Technical Service.

Table 5-1 Startup, Calibration, and Operating Error Messages (Continued)

5.1.2 Error Messages Preventing Operation

During initialization, calibration, and operation, the 2487 Detector may display the word <Error> on the Absorbance screen. An error of this type is usually fatal, preventing further operation of the detector and suspending the Absorbance display (Figure 5-1). Absorbance output is halted.

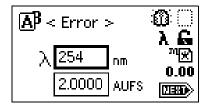


Figure 5-1 Fatal Error Display on the Absorbance Screen

When you encounter a fatal error, ensure that:

- There is no cuvette in the cuvette holder, and the empty cuvette holder is securely in place.
- The flow cell is clean.
- The left front panel door is shut securely.

Recycle power to the 2487 Detector. If the fatal error persists, contact Waters Technical Service.

<u>Table 5-2</u> lists the error messages that require you to contact Waters Technical Service if recycling power does not correct the error.

Table 5-2	Possibly	Fatal	Error	Messages
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Error Message	Description	Corrective Action
Electronic A/D failure	Lamp optimization is adjusted at the minimum level.	Recycle power.
	Acquisition via A/D converters is interrupt-driven. If interrupt is too long, problem with data acquisition is indicated.	Recycle power.
Method not found	Stored method data is invalid.	Recycle power to the 2487 Detector. This action removes the error.
Scan not found	Stored scan data is invalid.	Recycle power to the 2487 Detector. This action removes the error.
Configuration not found	Stored configuration data is invalid.	Recycle power to the 2487 Detector. This action removes the error.
Lamp data not found	Stored lamp data is invalid.	Recycle power to the 2487 Detector. This action removes the error.
Grating initialization failure: Backlash too high	Backlash is the difference between the forward and reverse peak positions of deuterium at 656 nm. If this difference is greater than 1 step, the unit displays this message.	Recycle power to the 2487 Detector. Contact Waters Technical Service.

Error Message	Description	Corrective Action
Electronic A/D failure	Data acquisition via A/D converters is interrupt-driven. If interrupt is too long, problem with data acquisition is indicated.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
System cannot respond	Error occurs while unit is positioning next wavelength or changing modes. Occurs during initialization or calibration.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Filter initialization failure: No reference energy	Unit sensors cannot find any light energy before homing the optical filter.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Filter initialization failure: No filters found	Unit sensors observe transition to dark before homing the optical filter.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Filter initialization failure: No response	Unit sensors cannot identify any dark regions.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Filter initialization failure: Shutter position	Unit sensors cannot find the shutter position.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Filter initialization failure: Order filter position	Unit sensors cannot find the order filter position.	Recycle power to the 2487 Detector. Contact Waters Technical Service.

Table 5-2 Possibly Fatal Error Messages (Continued)

Table 5-2	Possibly Fatal	Error Messages	(Continued)
			(

Error Message	Description	Corrective Action
Filter initialization failure: Erbium position	Unit sensors cannot find erbium filter position.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Grating initialization failure: No home sensor	Search for the home sensor failed.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
System not calibrated.	The calibration read from non-volatile memory is not valid.	Recycle power to the 2487 Detector. Perform manual calibration. Contact Waters Technical Service.
Communication failure: Reference A/D	A/D communication test failed.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Communication failure: Sample A/D	A/D communication test failed.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Dark current too low: 0	The dark energy level equals 0.	Recycle power to the 2487 Detector. Contact Waters Technical Service.
Dark current too high: <i>nnnnnn</i>	The dark energy level is above 1000000.	Recycle power to the 2487 Detector. Contact Waters Technical Service.

5.2 User-Selected Diagnostics

5.2.1 Overview

You may operate several diagnostics for use in:

- Troubleshooting the detector
- · Verifying the detector electronics and optics

To perform user-selectable diagnostics:

 Press the DIAG key from the 2487 Detector front panel. The detector displays the Diagnostics choice list (<u>Figure 5-2</u>).



Figure 5-2 Diagnostics Choice List

2. To access a specific diagnostic test, move the s or t arrow key to the diagnostic you want to run and press Enter, or press a number from 1 to 6 corresponding to the diagnostic number on the detector keypad. Choices that display other choices are indicated by >>.



Sticky diagnostics remain in effect until you disable them. When a sticky diagnostic is active, the 2487 Detector Absorbance screen displays a wrench icon (<u>Figure 5-3</u>).

- You can disable a specific sticky diagnostic by resetting it to the default settings.
- You can disable all active sticky diagnostics by pressing DIAG 1, Reset diagnostics.

If no sticky diagnostics are active, the wrench icon is removed from the Absorbance screen. When you power off the 2487 Detector, all sticky diagnostics are disabled.

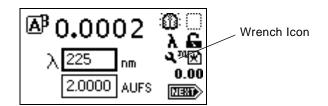


Figure 5-3 Absorbance Screen with Sticky Diagnostic Active

The user-selectable sticky diagnostics are:

- Fix (Set) Voltage Output
- Fix (Set) Absorbance Input
- Generate Test Peaks
- Optical Filter Override

Note: Application of sticky diagnostics affects results. To clear changes to the voltage output or absorbance input, or to make a manual optical filter change, select **1 Reset diagnostics** from the Diagnostics choice list, or reset power to the 2487 Detector.

<u>Table 5-3</u> lists the 2487 Detector diagnostics by their number on the choice list, followed by a brief description. See also <u>Section 5.2.2</u>, <u>Using the Diagnostics</u>, for more information.

Table 5-3 2487 Detector Diagnostics

Diagnostic	Description
1. Reset diagnostics	Resets all diagnostics to defaults. Removes sticky diagnostics and wrench icon.
2. Sample & ref energy	Allows you to view sample and reference energy (displayed in nanoAmps) on Channel A or B.

Diagnostic	Description
3. Input & output >>	List of diagnostics to control four contact closure inputs and two switch outputs: 1. Auto zero offset 2. Fix absorbance 3. Fix voltage 4. Contact closures & events 5. Previous choices <<
4. Lamp, display & keypad >>	List of diagnostics used to test lamp, display, and keypad functions: 1. Change lamp 2. Test keypad 3. Test display 4. Previous choices <<
5. Service	Diagnostics used by Waters Service personnel.
6. Other diagnostics >>	 Diagnostics that allow you to generate test peaks to check wavelength accuracy or to override the default filter setting: 1. Generate test peaks 2. Optical filter override 3. Previous choices <<

Table 5-3 2487 Detector Diagnostics (Continued)

5.2.2 Using the Diagnostics

The 2487 Detector employs both user-selectable and service diagnostics. You access user diagnostics by pressing the **DIAG** key. Only qualified Waters service personnel can access service diagnostics.

Contacting Waters Technical Service

If, after running the user diagnostics applicable to your problem and troubleshooting the detector (see <u>Section 5.3, Troubleshooting</u>), you are unable to correct an error condition, please contact Waters Technical Service at 1-800-252-4752, *U.S. and Canadian customers only.* Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

Using the Sample and Reference Energy Diagnostics

You use the sample and reference energy diagnostics to plot the output of the analog channels to examine noise fluctuations and to compare with the AU time trace. The current sample and reference energy readings are displayed in nanoAmps (nA). See <u>Figure 5-4</u>.

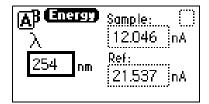


Figure 5-4 Sample and Reference Energy Diagnostic

To use the sample and reference energy diagnostic:

- 1. Press DIAG 2.
- 2. To change the wavelength, enter a new wavelength number.
- 3. Press Enter. When the new wavelength shifts to the left, the corresponding sample and reference energies are displayed.
- 4. If you are operating the detector in dual wavelength mode, press the A/B key to view sample and reference energy on the other wavelength.

Using the Input & Output Diagnostics

You use the Input & Output diagnostics to:

- Display and reset the autozero offsets.
- Fix (set) absorbance.
- Fix (set) the voltage on both the 2 V and 10 mV outputs.
- Monitor contact closures and toggle event switches.

- Generate test peaks.
- Override the optical filter.

To perform any of the Input & Output diagnostics, press **DIAG 3**, **Input & output**. A choice list appears (Figure 5-5).

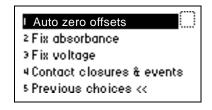


Figure 5-5 Input and Output Diagnostic Choice List

Displaying Auto Zero Offsets

From the Input & Output choice list, press **1**, **Auto zero offsets** (<u>Figure 5-6</u>). This diagnostic also allows you to display and clear the offset on both channels to zero by pressing **Cancel** (**Shift 0**).

Auto Zero Offsets
Channel A: 0.2131 AU
Channel B: 0.2131 AU
Press Cancel to reset to zero offset on both channels.

Figure 5-6 Auto Zero Offsets Diagnostic

Setting Fixed Absorbance Value

From the Input & Output choice list, press **2**, **Fix absorbance**, to set a fixed absorbance value for Channel A or Channel B. The allowable range is from –4.0000 AU to +4.0000 AU. For convenience, you may also specify sensitivity (AUFS) using this diagnostic (Figure 5-7). The allowable AUFS range is from +0.0001 to +4.0000 AUFS.

0	ce	bar	Abso	₿G	A
		AI	.0000		
	FS] AI	.0000		
	FS	A	.0000		

Figure 5-7 Fix Absorbance Diagnostic

This diagnostic sets the voltages on the analog output channels based on the current AUFS setting. This is a sticky diagnostic.

Setting Fixed Voltage Output

From the Input & Output choice list, press **3**, **Fix voltage**, to select a voltage for the analog output. You may select a voltage for both output channels in the -0.10 V to +2.10 V range (Figure 5-8).

AB Fiz Volt	age 🗍
2.00	V on 2V
10.00	mVon 10 mV

Figure 5-8 Fix Voltage Diagnostic

The voltage is driven on the selected analog channel (A or B). The 10 mV signal is scaled proportionally based on the entry you make in the 2 V field. This is a sticky diagnostic.

Monitoring Contact Closures and Setting Switches

 From the Input & Output choice list, press 4, Contact closures & events, to monitor the four contact closure inputs and to control the two switch outputs (Figure 5-9).

Input / Output)	<u> </u>
○ Chart mark ○ Lamp	SW1 🔤
○ Auto zero ○ Inject	S₩2 🞬

Figure 5-9 Contact Closures & Events Diagnostic

The Input & Output diagnostic allows real-time monitoring of the state of the contact closure inputs. A solid (filled in) circle indicates the contact closure is closed (ON = High). An open (empty) circle indicates the contact closure is open (OFF = Low).

- 2. For the outputs (SW1 and SW2):
 - a. Press Enter to display the active switch (surrounded by a dotted-line border).
 - b. Press any numerical key to change the status of the switch (ON to OFF or vice versa).
 - c. Press Enter to select the second switch.

Using the Lamp, Display, and Keypad Diagnostics

To access the lamp, display, and keypad diagnostic tests, press **DIAG 4**.

Using the Change Lamp Diagnostic



Attention: Make sure power to the 2487 Detector is off before you replace the lamp.

1. From the Lamp, display & keypad choice list, press **1**, **Change lamp**, to display the Change Lamp diagnostic (<u>Figure 5-10</u>).

Change Lamp Serial number of new lamp:			
123456789			
Date installed:			
Aug	\$	21	Vear 97

Figure 5-10 Change Lamp Diagnostic

2. Enter the 9-digit serial number and date of installation for the new lamp. Press **Enter** after each entry.

Use this function whenever you change the lamp to enter a new serial number and installation date. See <u>Section 4.4.2</u>, <u>Installing the New Lamp</u>, and <u>Section 4.4.3</u>, <u>Recording the New Lamp Serial Number</u>, for a complete explanation of the lamp replacement procedure.



Attention: If you do not record the new lamp serial number following the procedure in Section 4.4.2, Installing the New Lamp, and Section 4.4.3, Recording the New Lamp Serial Number, the lamp warranty is voided. The date of the previous lamp installation remains in the 2487 Detector memory.

Using the Test Keypad Diagnostic

1. From the Lamp, display & keypad choice list, press 2, Test keypad, to run the keypad test. A bitmap representation of the keypad is displayed (Figure 5-11).

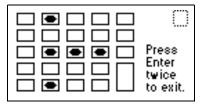


Figure 5-11 Keypad Diagnostic

2. Press any key to begin the test, then press each key until you have tried all of them. If the keypad is operating properly, each key location is filled in, then cleared with another press of the key. If any key does not respond when pressed, contact your Waters service representative.

Note: You must press *Enter* twice to exit the keypad diagnostic.

Using the Test Display Diagnostic

1. From the Lamp, display & keypad choice list, press 3, Test display, to run the display test.

When you press **3**, the display fills from top to bottom and from right to left, then returns to the Lamp, display & keypad choice list. If the display does not completely fill either horizontally or vertically, contact your Waters service representative.

2. From the Lamp, display & keypad choice list, press 4 to return to the diagnostics choice list.

Using the Other 2487 Detector Diagnostics

The user diagnostic screen provides two additional features. You can:

- Generate test peaks Use this diagnostic to specify the generation of test peaks to calibrate your chart recorder or data system.
- Manually override the optical filter Use this diagnostic to make a filter selection different from the detector's normal operating mode.

To operate either of these two diagnostics, press **DIAG 5**, **Other diagnostics** (Figure 5-12).

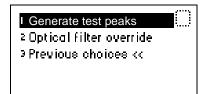


Figure 5-12 Other Diagnostics Choice List

Generating Test Peaks

 From the Other diagnostics choice list, press 1, Generate test peaks, to generate test peaks every 100 seconds on the chart, trace, or other output. A message indicates that test peaks are being generated (Figure 5-13).

Note: The Generate test peaks diagnostic works only in single-wavelength mode.



Figure 5-13 Generate Test Peaks Diagnostic

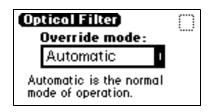
Every 100 seconds, the detector generates an approximate 1-AU peak with a standard deviation of 10 seconds on the trace, chart, or data system display until you disable the Generate test peaks diagnostic. The amplitude of the test peaks is affected by your choice of filter time constant.

This is a sticky diagnostic. When this diagnostic is enabled, the choice list changes to read "Disable test peaks."

 Press 1, Disable test peaks, from the Other diagnostics choice list, to turn this diagnostic off.

Overriding the Optical Filter

- ٩
- From the Other diagnostics choice list, press 2, Optical filter override, to manually override the detector's automatic filter choice. The Optical Filter Override diagnostic is displayed (Figure 5-14).



- Figure 5-14 Optical Filter Override Diagnostic
- 2. Press Enter to display the filter choice list. The choices are:

Automatic	1
Second Order	2
None	3
Erbium	4
Shutter	5

The 2487 Detector normally operates with the filter in the Automatic position. This is a sticky diagnostic.

3. Press the number corresponding to your filter choice or leave the default filter choice (Automatic) on.

This is a sticky diagnostic.

 To turn this diagnostic off, press DIAG 1 or select Automatic from the choice list.

5.2.3 Service Diagnostics

Only qualified Waters service personnel can access the 2487 Detector service diagnostics.

5.3 Troubleshooting

5.3.1 Introduction

This section provides some causes of errors and recommended troubleshooting actions. Keep in mind that the source of apparent detector problems may lie within the chromatography or your other instruments, as well as the 2487 Detector.

Most detector problems are relatively easy to correct. If you are unable to correct a problem or a failed condition, contact Waters Technical Service at 1-800-252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

When You Contact Waters

To expedite your request for service, have the following information available when you call Waters Technical Service:

- 2487 Detector serial number
- Problem symptom(s)
- Operating wavelength(s)
- · AUFS or measurement range
- · Flow rate
- · Filter setting
- Type of column
- · Operating pressure
- Solvent(s)
- System configuration (other components)

Note: The 2487 Detector may be configured as part of an Alliance[®] system with a 2690 Separations Module, the Millennium®³² Chromatography Manager, a PowerLine[™] controller, or a non-Waters product.

Diagnostics

The 2487 Detector performs some user-selected diagnostics to help you troubleshoot basic system problems. Refer to <u>Section 5.2.2</u>, <u>Using the Diagnostics</u>, for diagnostic descriptions and instructions on how to use them.

Error messages which may appear as you start up or operate the 2487 Detector and suggested corrective actions are described in <u>Table 5-1</u> and <u>Table 5-2</u>.

Power Surges

Power surges, line spikes, and transient energy sources can adversely affect detector operations. Be sure that the electrical supply used for the detector is properly grounded and free from any of these conditions.

5.3.2 Hardware Troubleshooting

This section contains general hardware troubleshooting for the 2487 Detector. See <u>Table 5-4</u>.

Symptom	Possible Cause	Corrective Action
Detector inoperative	Fuse blown	Check that the front panel display is operational; if not, replace the ac rear panel fuse.
	No power at outlet	Check outlet by connecting another electrical unit known to be in working order and see if it operates.
Front panel display fails to illuminate	Broken electrical connection	Check electrical connections.
	Fuse blown	Check and, if necessary, replace fuse(s).
	Bad LCD or control board	Contact Waters Technical Service.
Front panel displays odd characters	Faulty EPROMs Bad LCD control board	Contact Waters Technical Service.

Table 5-4 General System Troubleshooting

Table 5-4	General System	Troubleshooting	(Continued)
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Symptom	Possible Cause	Corrective Action
IEEE problems	Incorrect IEEE address	Set the correct IEEE address.
	Bad IEEE cable with correct IEEE address(es) set (2-29) (external)	Check the IEEE address. Replace the IEEE cable.
Keypad not functioning	Keypad defective	Recycle power to the 2487 Detector and run the keypad diagnostic. Contact Waters Technical Service.
Lamp life not calculated properly above 2300 hours	Revision 1.0 firmware in place.	Upgrade to Revision 1.01 firmware. Contact Waters Technical Service. With Revision 1.0 firmware, perform the Change Lamp diagnostic (Section 5.2.2, Using the Diagnostics) to reset lamp life to zero.
Deuterium lamp does	Faulty lamp	Replace the lamp.
not light	Lamp not plugged in	Plug in the lamp connector.
	Bad lamp power supply board	Contact Waters Technical Service.
	Lamp switch off	Check the rear panel connections (may be programmed to turn the lamp off) or timed event within a method (may be programmed to turn the lamp off).
No sample and reference energy	Lamp burned out	Attempt to reignite using the Lamp key. Replace the lamp.
	Lamp off	Check the lamp icon. Run the Sample & ref energy diagnostic.

Symptom	Possible Cause	Corrective Action
In dual wavelength mode, time scale is incorrect	Data rate on controller is > 1 pt./sec.	In dual wavelength mode under the control of ExpertEase and PowerLine systems, you must select a data sampling rate of 1 point per second.
Calibration or energy error on startup	Cuvette is installed, or a UV absorber in flow cell	Remove the cuvette. Flush the flow cell. Perform manual calibration.
Peak out of range error at start up	Cuvette in cuvette holder; mobile phase absorbance too high; air bubbles	Remove cuvette from cuvette holder. Be sure the flow cell mobile phase does not absorb above 250 nm. Ensure there are no air bubbles in the flow cell. Recalibrate the 2487 Detector. If problems persist, contact Waters Technical Service.
Analog output incorrect	AUFS setting changed	Reset the AUFS setting.

Table 5-4 General System Troubleshooting (Continued)

Appendix A 2487 Detector Specifications

This appendix lists individual operating specifications for the Waters[®] 2487 Dual λ Absorbance Detector, as shipped:

- <u>Table A-1</u> lists operational specifications.
- <u>Table A-2</u> lists optical specifications.
- <u>Table A-3</u> lists specifications for the optional flow cells.

Table A-1 Operational Specifications¹

Condition	Specification
Wavelength range	190 to 700 nm
Bandwidth	5 nm
Wavelength accuracy	<u>+</u> 1 nm
Wavelength repeatability	<u>+</u> 0.10 nm
Noise, single wavelength ²	< <u>+</u> 0.35 x 10 ⁻⁵ AU, dry cell, 254 nm, 1.0 sec. time constant (TC)
Noise, dual wavelength ^b	< <u>+</u> 2.5 x 10 ⁻⁵ AU, dry cell, 254 nm / 280 nm, 2.0 sec. time constant (TC)
Linearity ^b	5% at 2.5 AU, propyl paraben, 257 nm
Drift	1.0 x 10 ⁻⁴ AU/hour/° C at 254 nm (after 1 hour warmup)
Sensitivity setting range	0.0001 to 4.0000 AUFS

Table A-1 Operational Specifications¹ (Continued)

Condition	Specification			
Filter setting range	Single wavelength: 0.1 to 5.0 seconds, Hamming (default) 1 to 99 seconds, RC Dual wavelength: 1 to 50 seconds, Hamming (default) 1 to 99 seconds, RC			
Optical Component Spe	ecifications			
Lamp source	Deuterium arc lamp			
Flow cell	TaperSlit™ Flow Cell design			
Path length	10 mm (standard analytical)			
Cell volume	10 µL (standard analytical)			
Pressure limit	1000 psi / 70 bar			
Materials	316 stainless steel, fused silica, Teflon $^{^{(\!\!R\!)}}$			
Environmental Specific	cations			
Operating temperature	4 to 40 °C (39 to 104 °F)			
Operating humidity	20 to 80%, noncondensing			
Shipping and storage temperature	-40 to +70 °C (-40 to +158 °F)			
Shipping and storage humidity	20 to 80%, noncondensing			
Electrical Specifications				
Line frequency	50 to 60 Hz			
Line voltage	100 to 240 Vac			

Table A-1 Operational Specifications¹ (Continued)

Condition	Specification		
Fuse ratings	Two fuses: 100 to 240 Vac, 50 to 60 Hz F 3.15 A 250 V FAST BLO, 5 x 20 mm (IEC)		
Power consumption	145 VA (nominal)		
Two attenuated analog output channels: 2 VFS / 10 mVFS	Attenuation range: 0.0001 to 4.000 AU 2 V output range: -0.1 to +2.1 V 10 mV output range: -0.5 to +10.5 mV		
Two event outputs	Type:Contact closureVoltage: ± 30 VCurrent:1 A		
Four event inputs	Input voltage: <u>+</u> 30 V maximum 100 ms minimum period		
Dimensions			
Height	8.2 inches (20.8 cm)		
Length	19.8 inches (50.3 cm)		
Width	11.2 inches (28.4 cm)		
Weight	20.5 pounds (9.3 kg)		

¹ All specifications derive from instrument performance after a 1-hour warmup time.

²ASTM Standard E1657-94

Table A-2 Optical Specifications

Monochromator	Fastie Ebert Configuration
Grating	Plane holographic 1800 grooves/mm
Optical bandwidth	5 nm
Lamp power	30 W

Table A-3 Optional Waters TaperSlit Flow Cell Specifications

	Volume (μl)	Path Length (mm)	Inte Dian (Inc	e Tube ernal neter hes) Out	Pressure Rating psi/bar
Analytical Cell	10	10	0.009	0.009	1000/70
Semi-Prep Cell	2.6	3	0.040	0.040	1000/70
Microbore Cell	2.6	3	0.005	0.005	1000/70
Inert (titanium) Cell	10	10	0.010	0.010	1000/70
High Pressure Cell for LCMS	10	10	0.009	0.009	3000/210
Variable Path Length Flow Cell (VPF)		0.5 – 3 (factory preset at 0.5 mm)	0.04	0.04	1000/70

Appendix B Spare Parts

<u>Table B-1</u> lists the spare parts for the Waters[®] 2487 Dual λ Absorbance Detector. These spare parts are user-replaceable.

Table B-1 Spare Parts

Item	Part Number			
Fuses				
Fuse, 3.15 A, 5 x 20 mm	WAS163-16			
Fuse Holder	WAT055426			
Fuse, Lamp Power Supply (4A)	WAT071406			
Fuse, 2 A Micro (CPU)	WAS290423			
Optical Parts/Assemblies				
Deuterium Lamp Assembly/Preventive Maintenance Kit, 2487 Detector	WAS081142			
Analytical TaperSlit™ Flow Cell Assembly	WAS081140			
General Parts				
Waters Startup Kit, 2487 Detector	WAS081161			
Cuvette Holder Assembly	WAS081333			
Dual λ Absorbance Detector Wavelength Accuracy Cuvette Kit	WAT047690			
Dual λ Absorbance Detector Linearity Cuvette Kit	WAT047691			

Table B-1 Spare Parts (Continued)

Item	Part Number			
Two Cuvette Set (empty)	WAT047689			
Flow Cell Rebuild Kit, 10 mm	WAS081346			
Flow Cell Rebuild Kit, 3 mm	WAS081347			
MS Flow Cell Rebuild Kit	700000168			
MS Flow Cell Gasket Kit	700000169			
Gasket Kit, set of 10	WAS081348			
2487 Qualification Workbooks	WAT520-01			
Optional TaperSlit Flow Cells				
Analytical Cell	WAS081140			
Semi-Prep Cell	WAT081158			
Microbore Cell	WAT081159			
Inert (titanium) Cell	WAT081157			
High Pressure (3000 psi) Cell for LCMS	WAT081321			
Optional Variable Prep Flow Cells				
Variable Prep Flow Cell	700000923			
Variable Prep Flow Cell Quartz Rods and Seals Kit	700000688			
Variable Prep Flow Cell Adjust Screw Assembly Kit	700000689			
Variable Prep Entrance Lens Screw Assembly Kit	700000690			

Appendix C Warranty Information

This appendix includes information on:

- Limited express warranty
- · Shipments, damages, claims, and returns

C.1 Limited Express Warranty

Waters[®] Corporation provides this limited express warranty (the Warranty) to protect customers from nonconformity in the product workmanship and materials. The Warranty covers all new products manufactured by Waters.

Waters warrants that all products that it sells are of good quality and workmanship. The products are fit for their intended purpose(s) when used strictly in accordance with Waters' instructions for use during the applicable warranty period.

Limited Warranty

Waters Corporation warrants that the Waters 2487 Dual λ Absorbance Detector is a Class I medical device under 21 CFR 862.2260, as now in effect, and is for general purpose use and is not for use in clinical diagnostic procedures, and that during the Warranty period, the performance of all components of the Waters 2487 Dual λ Absorbance Detector [other than Third-Party Components (non-Waters named)], will not deviate materially from the Specifications for such detectors. Warranties, if any, that may be applicable to Third-Party Components shall be provided by the respective manufacturers or suppliers of such Third-Party Components, and Waters Corporation shall use reasonable efforts to assist Customer in securing the benefits of any such warranties.

Exclusions

The foregoing warranty does not apply to any material deviation from the Specifications by any component of the Waters 2487 Dual λ Absorbance Detector that results from (a) use of the Waters 2487 Dual λ Absorbance Detector for any purpose other than general-purpose use and specifically excluding use of the Waters 2487 Dual λ Absorbance Detector in clinical diagnostic procedures, or use of the Waters 2487 Dual λ Absorbance Detector for any on the Waters 2487 Dual λ Absorbance Detector in clinical diagnostic procedures, or use of the Waters 2487 Dual λ Absorbance Detector for investigational use with or without confirmation of diagnosis by

another, medically established diagnostic product or procedure, (b) errors or defects in any Third-Party Component, (c) modification of the Waters 2487 Dual λ Absorbance Detector by anyone other than Waters Corporation, (d) failure by Customer to install any Standard Enhancement in accordance with an update procedure, release of firmware or any operating system release, (e) any willful or negligent action or omission of Customer, (f) any misuse or incorrect use of the Waters 2487 Dual λ Absorbance Detector, (g) any malfunction of any information system or instrument with which the Waters 2487 Dual λ Absorbance Detector may be connected, or (h) failure to establish or maintain the operating environment for the Waters 2487 Dual λ Absorbance Detector in accordance with the operator's manual.

Exclusive Remedy

In the event of any failure of the Waters 2487 Dual λ Absorbance Detector to perform, in any material respect, in accordance with the warranty set forth herein, the only liability of Waters Corporation to Customer, and Customer's sole and exclusive remedy, shall be the use, by Waters Corporation, of commercially reasonable efforts to correct for such deviations, in Waters Corporation's sole discretion, replacement of the purchased Waters 2487 Dual λ Absorbance Detector, or refund of all amounts theretofore paid by Customer to Waters Corporation for the Waters 2487 Dual λ Absorbance Detector.

Disclaimers

THE LIMITED WARRANTY SET FORTH HEREIN IS EXCLUSIVE AND IN LIEU OF. AND CUSTOMER HEREBY WAIVES, ALL OTHER REPRESENTATIONS, WARRANTIES AND GUARANTEES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS OF THE WATERS 2487 DUAL λ ABSORBANCE DETECTOR FOR A PARTICULAR PURPOSE, INCLUDING FITNESS FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE. OR NONINFRINGEMENT, AND ANY WARRANTIES ARISING OUT OF COURSE OF DEALING OR COURSE OF PERFORMANCE. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT BECAUSE OF THE COMPLEX NATURE OF THE WATERS. 2487 DUAL λ ABSORBANCE DETECTOR AND ITS MANUFACTURE, WATERS CORPORATION CANNOT AND DOES NOT WARRANT THAT THE OPERATION OF THE WATERS 2487 DUAL λ ABSORBANCE DETECTOR WILL BE WITHOUT DEFECT. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT CUSTOMER IS SOLELY RESPONSIBLE FOR USE OF THE WATERS 2487 DUAL λ ABSORBANCE DETECTOR IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE.

Warranty Service

Warranty service is performed at no charge and at Waters' option in one of three ways:

- A service representative is dispatched to the customer facility.
- The product is repaired at a Waters repair facility.
- Replacement parts with appropriate installation instructions are sent to the customer.

Nonconforming products or parts are repaired, replaced with new or like-new parts, or refunded in the amount of the purchase price, when the product is returned. Warranty service is performed only if the customer notifies Waters during the applicable warranty period.

Unless otherwise agreed at the time of sale, warranty service is not provided by dispatching a service representative when the equipment has been removed from the initial installation location to a new location outside the home country of the selling company.

Warranty service is provided during business hours (8 AM to 5 PM, EST, Monday through Friday). Service is not available when Waters offices are closed in observance of legal holidays.

Warranty Service Exceptions

Warranty service is not performed on:

- Any product or part that has been repaired by others, improperly installed, altered, or damaged in any way.
- Products or parts identified prior to sale as not manufactured by Waters. In such cases, the warranty of the original manufacturer applies.
- Products that malfunction because the customer has failed to perform maintenance, calibration checks, or observe good operating procedures.
- Products that malfunction due to the use of unapproved parts and operating supplies.

Repair or replacement is not made:

- For expendable items such as gaskets, windows, lenses, and fuses, if such items were operable at the time of initial use.
- Because of decomposition due to chemical action.
- For used equipment.
- Because of poor facilities, operating conditions, or utilities.

Warranty Period

This instrument is warranted against defects in workmanship and materials for a period of 12 months (the "Warranty Period"), excluding assemblies, modules, serviceable parts, and components that may have different warranty periods. Refer to <u>Table C-1</u> for the warranty periods of such assemblies, modules, serviceable parts, and components.

The Warranty Period commences at the date of product shipment. The Warranty Period may be extended for such time, not to exceed one month, required to deliver and install the product at the customer's site. In no case does the Warranty Period extend beyond **13 months** from date of shipment. If an item is repaired or replaced during the Warranty Period, the replacement part or repair is warranted for the balance of the original warranty period.

Table C-1 summarizes the warranty periods for applicable components.

Table C-1 2487 Detector Warranty Periods

Item	Warranty
Waters 2487 Dual λ Absorbance Detector, excluding the components listed below	1 year
Normal Wear and Maintenance Parts (in Performance Maintenance Kit ¹ , part number WAS081142) Kit contains the source lamp ²	2000 hours

¹ Instrument improvements may result in changes to the contents of this kit.

 2 The 2487 Dual λ Absorbance Detector source lamp is warranted to light and pass startup diagnostic tests for 2000 hours or one year, whichever comes first.

Shipments

As all shipments are made Free On Board (FOB) shipping point, we suggest insurance be authorized on all shipments. Instruments and major components are packed and shipped via surface, unless otherwise required. Supplies and/or replacement parts are packed and shipped via United Parcel Service (UPS), UPS Blue, air parcel post, or parcel post unless otherwise requested.

Damages

The Interstate Commerce Commission has held that carriers are as responsible for concealed damage as for visible damage in transit. Unpack shipment promptly after receipt as there may be concealed damage even though no evidence of it is apparent. When concealed damage is discovered, cease further unpacking of the unit involved and request immediate inspection by local agent or carrier and secure written report of his findings to support claim. This request must be made within 15 days of receipt. Otherwise, the claim will not be honored by the carrier. Do not return damaged goods to the factory without first securing an inspection report and contacting Waters for a return merchandise authorization number (RMA).

Claims

After a damage inspection report is secured, Waters cooperates fully in supplying replacements and handling of a claim which may be initiated by either party.

Returns

No returns may be made without prior notification and authorization. If for any reason it is necessary to return material to Waters, please contact Waters Customer Service or your nearest Waters subsidiary or representative for a return merchandise authorization (RMA) number and forwarding address.

Appendix D Solvent Considerations



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

D.1 Introduction

Clean Solvents

Clean solvents provide:

- Reproducible results
- · Operation with minimal instrument maintenance

A dirty solvent can cause:

- · Baseline noise and drift
- · Blockage of the solvent filters with particulate matter

Solvent Quality

Use HPLC-grade solvents to ensure the best possible results. Filter through 0.45-µm filters before use. Solvents distilled in glass generally maintain their purity from lot to lot; use them to ensure the best possible results.

Preparation Checklist

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

- Filter solvents with a 0.45-µm filter.
- Degas and/or sparge the solvent.
- Stir the solvent.
- Keep in a place free from drafts and shock.

Water

Use water only from a high-quality water purification system. If the water system does not provide filtered water, filter it through a 0.45- μ m membrane filter before use.

Buffers

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

Tetrahydrofuran (THF)

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



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

D.2 Solvent Miscibility

Before you change solvents, refer to <u>Table D-1</u> to determine the miscibility of the solvents to be used. When you change solvents, be aware that:

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

When you switch from a strong buffer to an organic solvent, flush the buffer out of the system with distilled water before you add the organic solvent.

Table D-1	Solvent Miscibility
-----------	---------------------

Polarity Index	Solvent	Viscosity CP, 20 °C	Boiling Point °C (1 atm)	Miscibility Number (M)	λ Cutoff (nm)
-0.3	N-decane	0.92	174.1	29	
-0.4	Iso-octane	0.50	99.2	29	210
0.0	N-hexane	0.313	68.7	29	
0.0	Cyclohexane	0.98	80.7	28	210
1.7	Butyl ether	0.70	142.2	26	
1.8	Triethylamine	0.38	89.5	26	
2.2	Isopropyl ether	0.33	68.3		220
2.3	Toluene	0.59	100.6	23	285
2.4	P-xylene	0.70	138.0	24	290
3.0	Benzene	0.65	80.1	21	280
3.3	Benzyl ether	5.33	288.3		
3.4	Methylene chloride	0.44	39.8	20	245
3.7	Ethylene chloride	0.79	83.5	20	
3.9	Butyl alcohol	3.00	117.7		
3.9	Butanol	3.01	177.7	15	
4.2	Tetrahydrofuran	0.55	66.0	17	220
4.3	Ethyl acetate	0.47	77.1	19	260
4.3	1-propanol	2.30	97.2	15	210
4.3	2-propanol	2.35	117.7	15	
4.4	Methyl acetate	0.45	56.3	15, 17	260

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

Table D-1 Solvent Miscibility (Continued)

How to Use Miscibility Numbers

Use Miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent (<u>Table D-1</u>).

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

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

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

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

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

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

D.3 Buffered Solvents

When using a buffer, use a good quality reagent and filter it through a 0.45-µm filter.

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

D.4 Head Height

Position the solvent reservoirs at a level above the HPLC equipment or on top of the pump or detector (with adequate spill protection).

D.5 Solvent Viscosity

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

If the extent to which the pressure changes will affect the analysis is not known, monitor the pressure during the run using the Chart Out terminal.

D.6 Mobile Phase Solvent Degassing

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

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

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

D.6.1 Gas Solubility

Only a finite amount of gas can be dissolved in a given volume of liquid. This amount depends on:

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

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

Effects of Intermolecular Forces

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

Effects of Temperature

Temperature affects the solubility of gases. If the heat of solution is exothermic, the solubility of the gas decreases when you heat the solvent. If the heat of solution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in H₂O decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

Effects of Partial Pressure

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

D.6.2 Solvent Degassing Methods

This section describes the solvent degassing techniques that will help you to attain a stable baseline. Degassing your solvent also improves reproducibility and pump performance.

You can use either of the following methods to degas solvents:

- · Sparging with helium
- Vacuum degassing

Sparging

Sparging removes gases from solution by displacing dissolved gases in the solvent with a less soluble gas, usually helium. Well-sparged solvent improves pump performance. Helium sparging brings the solvent to a state of equilibrium, which may be maintained by slow sparging or by keeping a blanket of helium over the solvent. Blanketing inhibits reabsorption of atmospheric gases.

Sparging may change the composition of mixed solvents.

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Vacuum Degassing

The in-line vacuum degasser operates on the principle of Henry's Law to remove dissolved gases from the solvent. Henry's Law states that the mole fraction of a gas dissolved in liquid is proportional to the partial pressure of that gas in the vapor phase above the liquid. If the partial pressure of a gas on the surface of the liquid is reduced, for example, by evacuation, then a proportional amount of that gas comes out of solution.

Vacuum degassing may change the composition of mixed solvents.

D.6.3 Solvent Degassing Considerations

Select the most efficient degassing operation for your application. To remove dissolved gas quickly, consider the following.

Sparging

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

Vacuum Degassing

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

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

The in-line degasser is available as an option or factory-installed in the Waters[®] 2690 Separations Module, XE model.

D.7 Wavelength Selection

This section includes UV cutoff ranges for:

- Common solvents
- Common mixed mobile phases
- Chromophores

UV Cutoffs for Common Solvents

<u>Table D-2</u> shows the UV cutoff (the wavelength at which the absorbance of the solvent is equal to 1 AU) for some common chromatographic solvents. Operating at a wavelength near or below the cutoff increases baseline noise due to the absorbance of the solvent.

Solvent	UV Cutoff (nm)	Solvent	UV Cutoff (nm)
1-Nitropropane	380	Ethylene glycol	210
2-Butoxyethanol	220	Iso-octane	215
Acetone	330	Isopropanol	205
Acetonitrile	190	Isopropyl chloride	225
Amyl alcohol	210	Isopropyl ether	220
Amyl chloride	225	Methanol	205
Benzene	280	Methyl acetate	260
Carbon disulfide	380	Methyl ethyl ketone	330
Carbon tetrachloride	265	Methyl isobutyl ketone	334
Chloroform	245	Methylene chloride	233
Cyclohexane	200	<i>n</i> -Pentane	190
Cyclopentane	200	n-Propanol	210
Diethyl amine	275	n-Propyl chloride	225

Table D-2 UV Cutoff Wavelengths for Common Chromatographic Solvents

Solvent	UV Cutoff (nm)	Solvent	UV Cutoff (nm)
Dioxane	215	Nitromethane	380
Ethanol	210	Petroleum ether	210
Ethyl acetate	256	Pyridine	330
Ethyl ether	220	Tetrahydrofuran	230
Ethyl sulfide	290	Toluene	285
Ethylene dichloride	230	Xylene	290

Table D-2 UV Cutoff Wavelengths for Common Chromatographic Solvents (Continued)

Mixed Mobile Phases

<u>Table D-3</u> contains approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most commonly used. If you want to use a different concentration, you can determine approximate absorbance using Beer's Law, since absorbance is proportional to concentration.

Table D-3 Wavelength Cutoffs for Different Mobile Phases

Mobile Phase	UV Cutoff (nm)	Mobile Phase	UV Cutoff (nm)
Acetic acid, 1%	230	Sodium chloride, 1 M	207
Ammonium acetate, 10 mM	205	Sodium citrate, 10 mM	225
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
BRIJ 35, 0.1%	190	Sodium formate, 10 mM	200
CHAPS, 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoracetic acid, 0.1%	190

Mobile Phase	UV Cutoff (nm)	Mobile Phase	UV Cutoff (nm)
EDTA, disodium, 1 mM	190	TRIS HCI, 20 mM, pH 7.0, pH 8.0	202, 212
HEPES, 10 mM, pH 7.6	225	Triton-X™ 100, 0.1%	240
Hydrochloric acid, 0.1%	190	Waters PIC [®] Reagent A, 1 vial/liter	200
MES, 10 mM, pH 6.0	215	Waters PIC Reagent B-6, 1 vial/liter	225
Potassium phosphate, monobasic, 10 mM dibasic, 10 mM	190 190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

Table D-3 Wavelength Cutoffs for Different Mobile Phases (Continued)

Wavelength Selection for Chromophore Detection

Certain functional groups found in most compounds absorb light selectively. These groups, known as chromophores, and their behavior can be used to categorize the detection of sample molecules.

<u>Table D-4</u>^{*} lists some common chromophores, and their detection wavelengths (λ_{max}), as well as the molar absorptivity (ϵ_{max}) of each group. Use this information as a guide to select the optimal operating wavelength for a particular analysis. Because of the diversity possible within a given sample, scanning over a range of wavelengths may be necessary to determine the best wavelength for a particular analysis.

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Chromophore	Chemical Configuration	^λ max (nm)	[∈] max (L/m/cm)	^λ max (nm)	∈max (L/m/cm)
Ether	_0_	185	1000		
Thioether	—S—	194	4600	215	1600
Amine	—NH ₂	195	2800		
Thiol	—SH	195	1400		
Disulfide	—S—S—	194	5500	255	400
Bromide	—Br	208	300		
lodide	—I	260	400		
Nitrile	—C≡N	160	_		
Acetylide	—C≡C—	175-180	6000		
Sulfone	—SO ₂ —	180	_		
Oxime	—NOH	190	5000		
Azido	>C=N—	190	5000		
Ethylene	—C=C—	190	8000		
Ketone	>C=O	195	1000	270-285	18-30
Thioketone	>C=S	205	strong		
Esters	-COOR	205	50		
Aldehyde	—СНО	210	strong	280-300	11-18
Carboxyl	—СООН	200-210	50-70		
Sulfoxide	>S—0	210	1500		
Nitro	-NO ₂	210	strong		
Nitrile	-ONO	220-230	1000-2000	300-400	10

Table D-4 Electronic Absorption Bands of Representative Chromophores

Chromophore	Chemical Configuration	^λ max (nm)	[∈] max (L/m/cm)	^λ max (nm)	∈max (L/m/cm)
Azo	—N=N—	285-400	3-25		
Nitroso	—N=O	302	100		
Nitrate	-ONO ₂	270 (shoulder)	12		
Allene	—(C=C)2— (acyclic)	210-230	21,000		
Allene	(C=C)3	260	35,000		
Allene	(C=C)4	300	52,000		
Allene	(C=C)5	330	118,000		
Allene	—(C=C)2— (alicyclic)	230-260	3000-8000		
Ethylenic/ Acetylenic	C=C—C=C	219	6,500		
Ethylenic/Amido	C=C—C=N	220	23,000		
Ethylenic/ Carbonyl	C=C—C=O	210-250	10,000-20,000		
Ethylenic/Nitro	C=C-NO ₂	229	9,500		

Table D-4 Electronic Absorption Bands of Representative Chromophores (Continued)

^{*}Willard, H. H. and others. *Instrumental Methods of Analysis*, 6th ed. Litton Educational Publishing, Inc., 1981. Reprinted by permission of Wadsworth Publishing Co., Belmont, California, 94002.

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