

# DYE QUALITY CONTROL REFERENCE MATERIAL KIT

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## I. INTRODUCTION

The Quality Control Reference Materials (QCRM) portfolio is a unique collection of standards and mixtures. These products allow users to evaluate and benchmark the chromatography system before analysis of critical material. The products in the portfolio are all precisely formulated based on the expertise of Waters scientist.

The AutoPurification™ Dye QCRM contains 3 compounds including Thionin, Thioflavin T, and Crystal Violet.

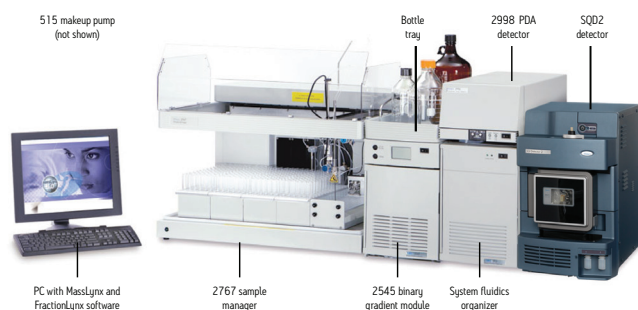
This standard mix is used to confirm the benchmark performance of a Preparative/Purification System through fraction collection. This particular QCRM is precisely formulated to allow the user to verify correct fraction collection. This test is highly valuable to perform upon first use/installation, and any time the system has not been used or has had any changes. These compounds were vigorously tested and evaluated and chosen because they provide the following advantages:

- Well-separated
- Easily visually identified

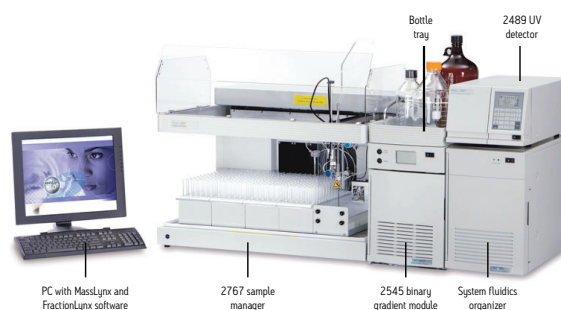
### a. MS-Directed Purification

The Waters MS-directed autopurification system uses the Waters SQD2 Mass Detector as its primary detector.

*MS-Directed System*



*UV-Directed System*



## b. The Purpose of a Quality Control Reference Material

Waters recommends to benchmark a purification chromatographic system with a QCRM prior to system usage when there is confidence that the system is in good working order. It is recommended to run and save the initial results and to continue to compare new QCRM results to the previous benchmark before any critical assay is run, and after any hardware, column, or mobile phase changes.

The QCRM standard benchmark result will be specific to the performance of the system it is run on. All chromatographic systems have some minor level of variability from run-to-run. Trending of results over time is useful for defining variability on a single system, multiple systems or on systems in different locations.

Setting specification for QCRM results should not be done without sufficient data trending. Once variability is understood, QCRM results will help determine the capability of the system to provide reliable results.

## c. What Affects Your Quality Control Reference Material Results

The goal of the QCRM specifications and criteria will be to indicate that the system is functioning as expected. The system is comprised of many interdependent components working together to produce results within an expected specification. An issue in any one of the components can affect your QCRM result. Variability can be attributed to hardware, software or chemical changes. All components performing correctly will produce results within an expected variability.

Sources of the variability that may affect system results include:

- Mobile phase preparation
- Column performance
- Tubing size
- System component performance (pump, injector, detector)
- Temperature control
- Data collection rate
- Integration

Differences in any of the components mentioned can result in system to system variability of results even when each system's components are functioning correctly.

## II. STORAGE AND STABILITY

The AutoPurification™ Dye QCRM contains 3 compounds including Thionin, Thioflavin T, and Crystal Violet. The compounds are stable in their original packaging, through the expiration date listed as provided in 10 mL amber ampule before opening. This is intended to be a one time use product. The integrity of the standard can not be guaranteed if stored after first use.

## III. RECOMMENDED USAGE OF THE DYE QCRM

### a. Testing System Performance

After installing FractionLynx™, it is recommended to test the system to confirm that the instruments are functioning correctly, and that the system produces the expected chromatographic results.

Testing involves using the Dye Kit (part number 716000765) to inject dyes and subsequently collect them. A passing result for this system-level test means that the system instruments function properly. If the test results differ from those in the examples provided, ensure that collection parameters are set appropriately.

The conditions defined in each of the following tests are designed to help rapidly determine whether all the components function as a system. Actual system performance (column load per run) depends greatly on conditions such as the HPLC solvents or modifiers, and the dimension of the columns.

For the MS-directed system test and the UV-directed system test, many FractionLynx method parameters are identical, including the gradient and UV detector parameters. The chromatogram examples represent results that can be expected, although they can differ somewhat from system to system.

### b. Performing the Delay Timing Test Manually

Determine the delay time manually by injecting the dye standard from the dye kit. The following test is conducted using a Waters 19 mm x 50 mm XTerra® column.

**Note:** When using a different size and type of column than the one described here, the flow and injection volumes must be adjusted accordingly. Pressure balancing and flow to the mass spectrometer need to be performed at the purification flow rate. When using the column isocratically, the retention time is approximately 90 to 120 seconds, based on the column size and flow rate.

Required materials:

- Stopwatch
- Mobile phases:
  - A Water/TFA, 0.1% (v:v)
  - B Acetonitrile/TFA, 0.1% (v:v)
- Sample dye kit, part number 716000765

Table 1. Test Dye Compounds Information

Item	Thionin Acetate	Thioflavin T	Crystal Violet
m/z	227.1	282.1	371.2
UV <sub>max</sub>	590 nm	418 nm	590 nm
Formula	C <sub>12</sub> H <sub>9</sub> N <sub>3</sub> SC <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	C <sub>17</sub> H <sub>19</sub> ClN <sub>2</sub> S	C <sub>25</sub> H <sub>3</sub> OCIN <sub>3</sub>

#### To set up the delay timing test:

1. In the FractionLynx method, set the split collector delay to 30 seconds.
2. In MassLynx™, create an inlet method with the following settings:
  - Run at the required flow rate for 3 minutes with 10% A and 90% B.

For UV-directed systems, set the UV detector to the following parameters, and ensure that the connections on the UV Fraction Manager correspond to the flow rate used for this test:

- Waters 2998 PDA Detector to scan from 200 to 600 nm
- Waters 2489 UV Detector to monitor 418 nm and 590 nm.

3. For MS-directed systems, create an MS method for a 3 minute scan acquisition with electrospray + ve, and specify these parameters:
  - Centroid = 150 to 550 amu scan for 0.5 seconds
  - Inter-scan delay = 0.1 seconds
  - Cone Voltage = 35 V.

#### To run the delay timing test

1. In the MassLynx sample list, specify these values:
  - Specify the Injection Volume as 100 µL.
  - Enter these values depending upon the detectors configured in the system:
    - For an MS-directed system, enter 227.1 in the Mass A column, 282.1 in the Mass B column, and 371.2 in the Mass C column. In the Fraction Trigger 1 column, enter Mass A. In the Fraction Trigger 2 column, enter Mass B. In the Fraction Trigger 3 column, enter Mass C.
    - For a UV-directed system, enter 418 in the Wavelength A column and 590 in the Wavelength B Column. In the Fraction Trigger 1 column, enter Wavelength A for a PDA detector or UV1 for a UV detector, such as a Waters 2489. In the Fraction Trigger 2 column, enter Wavelength B for a PDA detector or UV2 for a UV detector.

*Tip: UV1 and UV2 correspond to Channel A and Channel B wavelengths in the Inlet method. The setting in the Wavelength column is for reporting purposes only; it does not control the 2489 detector. The software uses the value set in the Fraction Trigger column, UV 1, UV 2, against the value set in the Inlet Editor.*

2. When the injection occurs, monitor the waste line from the fraction collector valve. As soon as the dye appears in the waste line of the fraction collector valve, start the stopwatch.

When you hear the fraction collector valve click to the dispense position, stop the stopwatch. The dye should have eluted before the collection valve opened.

4. Calculate the actual Splitter/Collector delay as follows:

The actual delay is equal to original Splitter/Collector delay specified in the FractionLynx method (e.g., 30 seconds) minus the time recorded on the stopwatch.

Example: If the dye is seen in the waste line 15 seconds before the valve clicks to the collection position, the delay time is calculated as follows:

Actual splitter collector delay = 30 – 15 = 15 seconds.

5. In the Timing tab of the FractionLynx method, change the Splitter/Collector delay to the value just calculated, and rerun the experiment to ensure that the delay time is correct.
6. Repeat the procedure for all flow rates, such as 10, 15, 20, or 25 mL/min.
7. For the MS-based system, complete the procedure for APCI and ESI if you are using both probes.
8. Perform a chromatography test of the entire system, as described below.

### c. Performing a Chromatography Test

To perform the chromatography test, an inlet method using the parameters provided in this procedure needs to be created. The XTerra 19 mm x 50 mm column is used to perform the tests in the manual. Scale the method appropriately for different flow rates and/or differently-sized columns. Use the flow rate that will be used for purification.

To create an inlet method for the system test:

1. Set up the Inlet Editor to run a 10 minute method labeled “Dye.”
2. Set up the Waters 2545 binary gradient module to use these parameters:
  - Mobile Phase A: Water with 0.1% TFA or 0.2% formic acid
  - Mobile Phase B: Acetonitrile with 0.1% TFA or 0.2% formic acid
  - Flow Rate: specify the same rate that you used in the delay timing test.
  - Run Time: 10 minutes
  - Gradient conditions as specified in Table 2

Table 2. Gradient Conditions for the System Test

Time	A%	B%	Curve Number
T = 0 minutes	95	5	6
T = 1 minutes	95	5	6
T = 7 minutes	30	70	6
T = 7.5 minutes	5	95	6
T = 8.5 minutes	5	95	6
T = 9 minutes	95	5	6
T = 10 minutes	95	5	6

3. In the Inlet Editor, set the wavelength range you want to acquire, based on your detector type:
  - For a Waters 2998 PDA Detector, in the Inlet Editor, set the wavelength range to 210 nm to 650 nm.
  - For a Waters 2489 dual wavelength absorbance detector, in the Inlet Editor, set the single channel wavelength to 418. Set the dual channel wavelength to 590.
4. For an MS-directed system, create a mass spectrometer method with these parameters, and then set it to run for 10 minutes:
  - Set up a Scan Function: 150 to 500 amu
  - ES+
  - Centroid Data
  - 0.5 second scan time with a 0.1 second Inter Scan Delay
  - Cone Voltage = 35 V
5. In the MassLynx sample list, perform these tasks:
  - Specify an injection volume of 100 µL.
  - For an MS-directed system, enter 227.1 in the Mass A column, 282.1 in the Mass B column, and 371.2 in the Mass C column. In the Fraction Trigger 1 column, enter Mass A. In the Fraction Trigger 2 column, enter Mass B. In the Fraction Trigger 3 column, enter Mass C.

- For a UV-directed system, enter 418 in the Wavelength A column and 590 in the Wavelength B column. In the Fraction Trigger 1 column, enter Wavelength A for a PDA detector or UV1 for a UV detector, such as the Waters 2489 dual wavelength absorbance detector. In the Fraction Trigger 2 column, enter Wavelength B for a PDA detector, or UV2 for an UV detector.

*Tip: The Mass and Wavelength columns are for information only, unless the corresponding fraction information is entered in the Fraction Trigger column.*

- For systems that include a ZMD mass detector, set the instrument's multiplier voltage to 550 V. For systems that include a ZQ™ mass detector, set the multiplier voltage to 500 V. For systems that include a 3100 or SQD2 mass detector, retain the default gain setting of 0.1.
- From the shortcut bar, click FractionLynx > Collection Control, and verify that your system is activated.

**To set the FractionLynx method parameters for a system test**

- In the FractionLynx main window, click Edit FractionLynx method.
- In the FractionLynx method, click each tab to set the parameters you want to define for this fraction collection method.

Exception: The FractionLynx method parameters for the UV-based system test apply to Waters UV detectors. When using the detectors of other manufacturers, the threshold values can differ slightly. They might also differ from system to system. Use the values specified in the Table 3 as guidelines.

Table 3. FractionLynx Method Settings

Parameter Tab	Settings You Can Specify
General	<ul style="list-style-type: none"> <li>Set Fraction Collection to On and Peak Type to Preparative</li> <li>Select the Max Fractions per injection check box, and specify a value of 10</li> <li>Select the Max Tubes per injection check box, and specify a value of 10</li> <li>Rinse time = 0</li> <li>Span = 0.5 amu</li> </ul>
Timing	<ul style="list-style-type: none"> <li>Solvent Front Delay = 0 seconds</li> <li>Split/Collector Delay = seconds, where x = the split collector delay determined earlier</li> </ul>
Volume	<ul style="list-style-type: none"> <li>Minimum Fraction Width = 3 seconds</li> <li>Maximum Fraction Width = 120 seconds</li> <li>Maximum Tube Fill = 90 percent</li> </ul>

- Based on your detector, enter the values specified in the table below.

*Tip: Where values are not specified in Table 4, retain FractionLynx method's defaults.*

Table 4. FractionLynx Method Settings Based on Detector Type

Detector Type	FractionLynx Method Tab	Settings You Can Specify
2489	UV	<ul style="list-style-type: none"> <li>MIT = 1000</li> <li>Peak Start = Leading Edge Gradient % with a value of 30</li> <li>Terminate Peak = Below Gradient % with a value of 60</li> </ul>
PDA	PDA	<ul style="list-style-type: none"> <li>Span = 3 nm</li> <li>MIT = 5,000</li> <li>Peak Start = Leading Edge Gradient % with a value of 30</li> <li>Terminate Peak = Below Gradient % with a value of 60</li> </ul>
Mass Spec	ES+	<ul style="list-style-type: none"> <li>ES+ Ion Adducts = 1</li> <li>MIT = 5,000,000</li> <li>Peak Start = Use MIT only</li> <li>Terminate Peak = Use MIT only</li> </ul>
Analog	Analog	<ul style="list-style-type: none"> <li>MIT = 5,000</li> <li>Peak Start = Leading Edge Gradient % with a value of 30</li> <li>Terminate Peak = Below Gradient % with a value of 60</li> </ul>

1. From the shortcut bar, click FractionLynx > Collection Control, and verify that your system is activated.
2. From the MassLynx toolbar, click Start.

### UV Chromatography Dye Test Results

These chromatograms represent typical results of the dye test. The chromatograms show three separate color samples of blue, yellow, and violet collected into separate vials labeled 1:16, 1:17, and 1:18 to 1:19.

**Requirements:** Sample dye kit, which contains three compounds for testing the ability of the AutoPurification System to perform high-flow-rate targeting and fractionation of specified masses and wavelengths.

The compounds elute in this order:

- Thionin acetate loses the acetate salt ( $C_2H_4O_2$ ) to give this [M+H] ion:

Thionin ([M+H] = 228.1), lambda max. = 590 nm

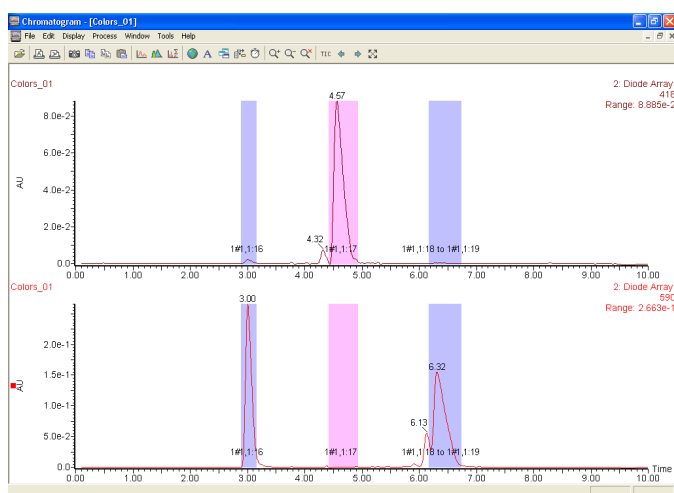
- Thioflavin T loses a Cl to give this [M+H] ion:

Thioflavin T ([M+H] = 283.1), lambda max. = 418 nm

- Crystal violet loses HCl to give this [M+H] ion:

([M+H] = 372.2), lambda max. = 590 nm

*Tip: Report masses to one decimal place.*

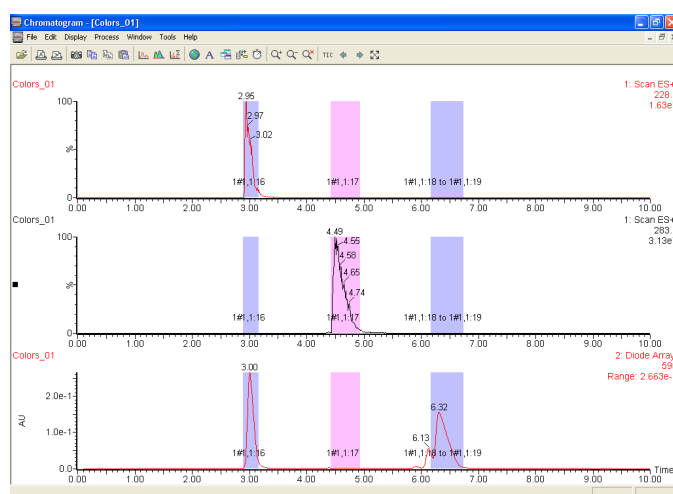


*This sample was collected using the Waters 2998 PDA Detector. The wavelength values were put in the sample list, and the fraction triggers were set to Wavelength A and B.*

- The extracted wavelength shows the correct picture of what was actually collected.
- Click Display > Wavelength to input the wavelengths of choice, and then click OK to get the extracted wavelengths.
- To extract the spectrum, double-click the peak in the Total Absorbance Chromatogram (TAC).

Actual results can vary if different column sizes, flow rates, and injection volumes are used.

### MS Chromatography Dye Test Results



*The chromatograms above represent typical results of the dye test. The three components of the dye mix have been separated and collected into separate vials labeled 1:16, 1:17, and 1:18 to 1:19.*

The extracted ion shows the correct picture of what was actually collected into each vial. Double-click the peak in the Total Ion Chromatogram (TIC) to extract the spectrum, and double-click the spectrum of the mass of interest to extract the mass chromatograms. Always consider the extracted ion or wavelength when troubleshooting as it will give the correct representation of what was actually collected.

## IV. TROUBLESHOOTING

For specific troubleshooting of the system and settings refer to the AutoPurification System Guide 71500135202ra.

## V. ORDERING INFORMATION

To order these products, contact your nearest subsidiary, or visit [www.waters.com](http://www.waters.com) and click on **Order Center**.

Description	Part Number
Auto Purification Dye Quality Control Reference Material Kit	<a href="#">716000765</a>

### Related Products

Description	Part Number
XBridge™ C <sub>18</sub> , 5 μm, 19 x 50 mm	<a href="#">186002977</a>
XSelect™ CSH C <sub>18</sub> , 5 μm, 19 x 50 mm	<a href="#">186005420</a>
XSelect CSH Phenyl-Hexyl, 5 μm, 19 x 50 mm	<a href="#">186005446</a>
XSelect CSH Fluoro-Phenyl, 5 μm, 19 x 50 mm	<a href="#">186005433</a>
XTerra MS C <sub>18</sub> , 5 μm, OBD 19 x 50 mm	<a href="#">186001930</a>
Symmetry® C <sub>18</sub> , 5 μm, OBD 19 x 50 mm	<a href="#">186000210</a>
Atlantis® T3 C <sub>18</sub> , 5 μm, OBD 19 x 50 mm	<a href="#">186003696</a>

Thank you for choosing a Quality Control Reference Material (QCRM) from Waters. The standards are manufactured in our ISO 9001, ISO 17025 facility. Each standard is manufactured to ensure optimal reproducibility from lot-to-lot. A Waters QCRM can be depended on for its' accuracy. This removes one variable from your system variability and provides you the most dependable starting point for your testing. If the QCRM box shows significant damage, notify the carrier and your supplier at once and retain evidence of shipping damage so that a claim can be made.

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