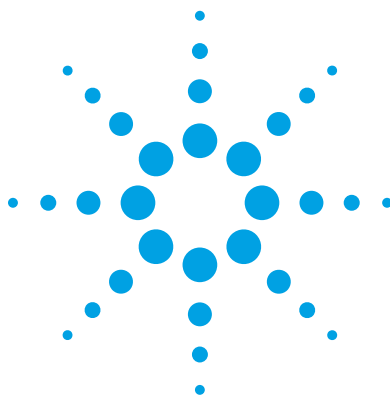




**Agilent G9530A
RapidFire 365
High-throughput Mass
Spectrometry System**

User Guide



Agilent Technologies

Notices

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Software Revision

This guide is valid for the RF-MS 4 revision of the RapidFire Control Software.

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CAUTION

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WARNING

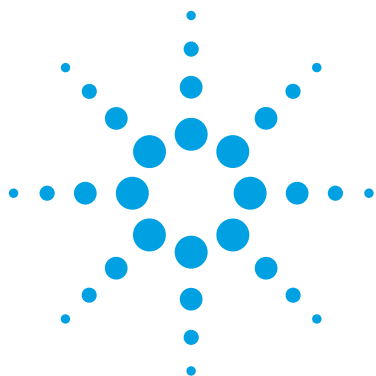
A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

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System Preparation

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This chapter contains information to help you start up and turn off the Agilent RapidFire 365 High-throughput Mass Spectrometry System for daily use.

What is the RapidFire 365 High-throughput Mass Spectrometry System?

The RapidFire 365 High-throughput Mass Spectrometry System helps to eliminate bottlenecks in your drug discovery workflow and enhance the screening process of sample analyses. By feeding samples directly to the mass spectrometer, RapidFire technology enables the acceleration of drug discovery pipeline decisions.

The high-throughput mass spectrometry system consists of a proprietary sample purification and injection system that interfaces directly with the mass spectrometer. The RapidFire-MS system can simultaneously analyze



multiple analytes in complex mixtures presented by biological samples and remove contaminants such as salts, buffers, detergents, proteins, microsomes, and subcellular material.

The RapidFire system facilitates the analysis of one 384-well plate in about 50 minutes or as many as ten 384-well plates (3840 individual samples) in a single instrument shift (8 hours). When you run an experiment continuously over a weekend, the system can run 63 plates (24192 individual samples). The throughput of the RapidFire system for label-free biologically-relevant data approaches that of optical technologies.

The dimension of the RapidFire system is 170 cm × 155 cm × 81 cm. It weighs 475 kg. The system is equipped with one BenchBot sample handler and four plate stacks. The plate stacks enable the system to process 63 micro plates in one batch. Three plate stacks serve as the input stacks, and each can accommodate 21 micro plates. One plate stack is left empty to serve as the output stack. When all of the plates from one input stack is moved to the output stack, the original input stack becomes the output stack. In addition, the barcode scanner is capable of reading barcode labeled on any side of the sample plates.

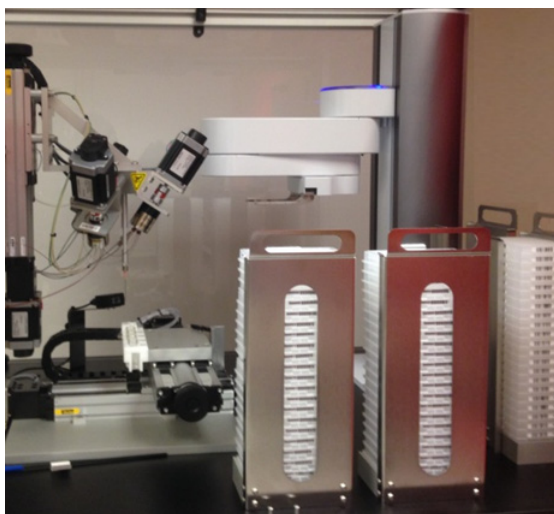


Figure 1 BenchBot sample handler and input stacks.

To prepare solvents

Only use LCMS-grade, filtered, degassed solvents. Change solvents according to your assay.

- 1 Pull out the solvent drawer.



- 2 Check that you have enough solvent for the runs that you plan to make and fill the following containers accordingly.
 - Lines 1A, 1B, 1C, and 1D feed Pump 1
 - Lines 2A, 2B, 2C, and 2D feed Pump 2
 - Lines 3A, 3B, 3C, and 3D feed Pump 3
 - Water in bottle labeled 100% water
 - Acetonitrile in bottle labeled 100% acetonitrile. Other organic solvents, such as methanol, can also be used.

WARNING

Handle solvents safely as described in the *Safety Guide*.

- 3 Gently shake the filters and whirl the flasks around to remove potential air bubbles.

1 System Preparation

To check waste capacity

To check waste capacity

- 1 Check that the waste flasks have enough room to accommodate the waste that will be generated from the runs that are planned.
- 2 If more capacity is needed:
 - a Empty the waste carboy where the two green RapidFire System waste lines are directed.
 - b Empty the vacuum waste flask.
 - c Check that the two green RapidFire System waste lines are not dipping in waste fluid, but merely sticking out of the lid of the carboy.

WARNING

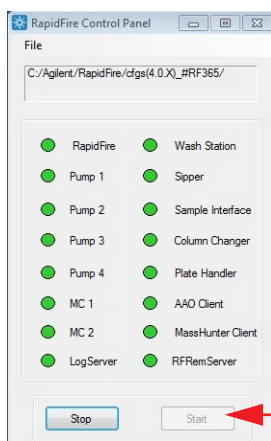
Handle waste solvents safely as described in the *Safety Guide*.

To start the vacuum system

- 1 Open up the vacuum valve or switch on your stand-alone vacuum pump.

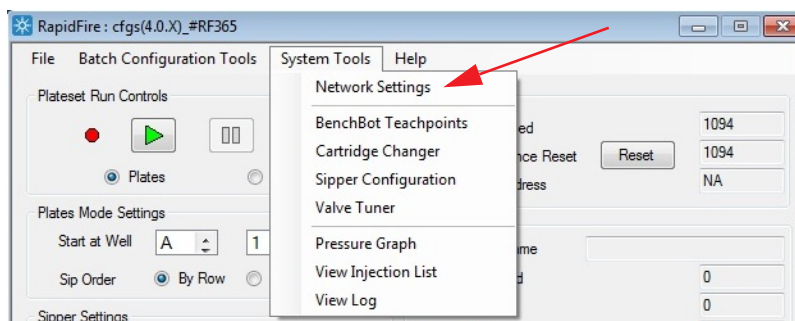
To start the software

- 1 Double-click the **RapidFire Control Panel** desktop icon.
- 2 Click **File > Choose CFGs**.
- 3 Select the configuration folder under **C:\Agilent\RapidFire**.
- 4 Click **Start** in the Control Panel dialog box.



The icons turn green as the system components start up.

- 5 Double-click the **RapidFire UI** desktop icon.
- 6 Click **System Tools > Network Settings**.



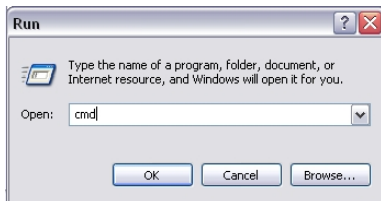
- 7 Verify that the IP addresses for the RapidFire System computer and the mass spectrometer computer are correct.

1 System Preparation

To home the cartridge changer and replace the cartridge

Tip To find the IP address:

- a** Click **Run** on the Windows Start menu.
- b** When the run dialog box opens, type **cmd** and click **OK**.

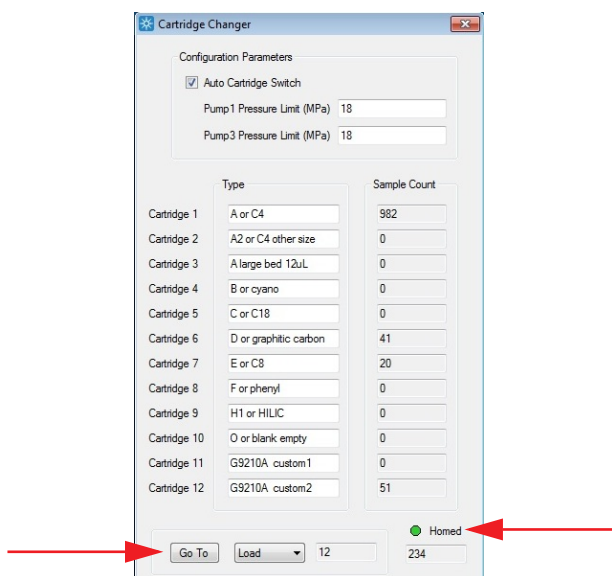


- c** When a command window opens, type **ipconfig -all** and press **Enter**.
The IP address is displayed in the window.
- 8** To connect to the program server, click **Connect** in the main window.
The icon next to the **Connect** button turns green.

To home the cartridge changer and replace the cartridge

- 1** To open the Cartridge Changer dialog box, click **System Tools > Cartridge Changer**.
- 2** If the **Homed** icon is red, home the device as follows:
 - a** Click **Home** in the drop-down menu.
 - b** Click **Go To**.
The **Homed** icon turns green.

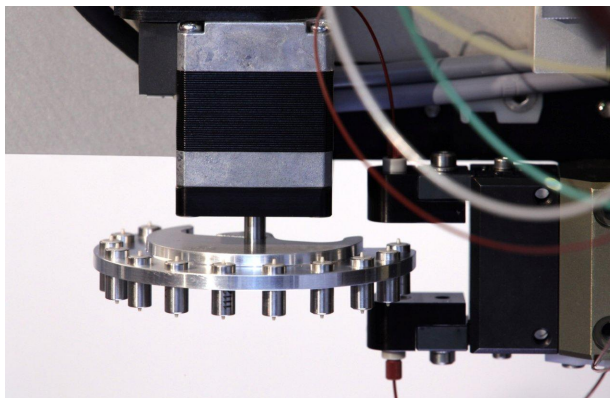
To home the cartridge changer and replace the cartridge



Be sure that a cartridge is present in the Cartridge 1 slot, as the cartridge changer travels to this position after the homing routine is complete.

3 To load up to 12 new cartridges in the holder:

- a** Click **Load** in the drop-down menu.
- b** Click **Go To** to get access to the slots.



1 System Preparation

To home the stages

- c After removing their protective packaging, insert the new cartridges in the holder, with the notch end facing up.
- 4 Update the information in the Cartridge Changer dialog box:
 - a Enter the type for each new cartridge.

See “[RapidFire System Cartridges](#)” on page 83 for a list of columns that are available for the RapidFire System.
 - b To reset the **Sample Count** of a particular cartridge to zero or any given non-zero value, double-click the corresponding field and confirm your input.
 - c Click **Update**.

To home the stages

Use this procedure to calibrate the RapidFire System positions in any of the following situations:

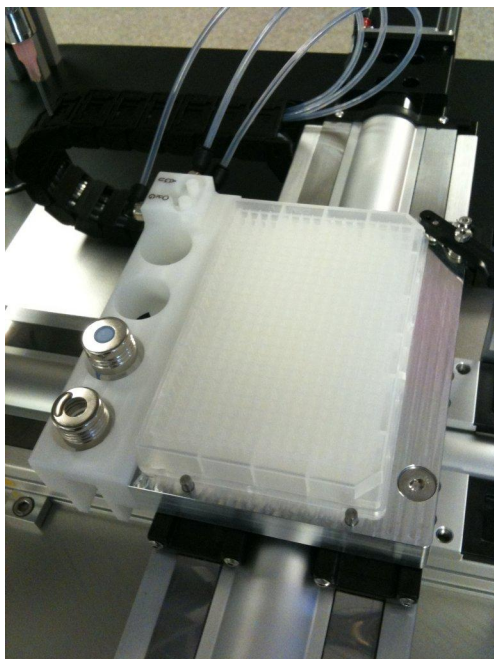
- When you replace the sipper tube or its pink guiding needle.
- When you teach a new type of plates, such as round instead of conical bottom.

Before you begin For this calibration protocol, you need an empty example plate in either the 96- or 384-well format.

- 1 To open the Sipper Configuration wizard, click **System Tools > Sipper Configuration**.
- 2 Click **Home** in the wizard and wait until all three (x,y,z) stages have found their home positions (0,0,0).
- 3 If your experiments use the same types of plates as the types last used to calibrate the RapidFire System, then:
 - a Close the wizard.
 - b Continue with “[To check the valves](#)” on page 18.Otherwise, continue with Steps 4-12 to set a sipper safe height.
- 4 When the Sipper Safe Height window appears, select the plate type to configure from the list:



- 5 Place an empty plate on the holder between the metal pins. Use the same plate type as you are using for your experiment.



- a Manually adjust the stage to clear the plate, wash station, and matrix bottles by about 0.5 cm or 1/4".
- b Click **Set**, then **Next**, in the software wizard.

Tip To move the stage manually, first disengage the power in *either* of the following ways:

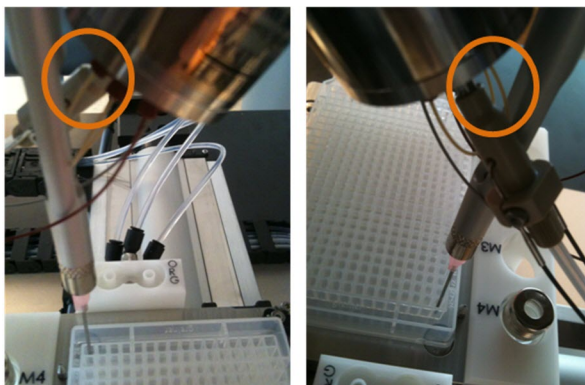
- Open one of the interlocked doors, *or*
- Press the **E-STOP** button.

1 System Preparation

To home the stages

Then turn the stage knobs until the stage reaches the desired position. Finally, close the doors or release the **E-STOP** button to restore power.

- 6 When the Aqueous Wash Station Position window appears:
 - a Manually adjust the stage so that the sipper tube is deep within the aqueous wash station chimney, but does not contact the walls or bottom.
 - b Click **Set**, then **Next**.
- 7 When the Organic Wash Station Position window appears.
 - a Manually adjust the stage so that the sipper tube is deep within the organic wash station chimney, but does not contact the walls or bottom.
 - b Click **Set**, then **Next**.
- 8 When the Middle (Trough) Wash Station Position window appears:
 - a Manually adjust the stage so that the sipper tube is within the central drain of the wash station.
 - b Set the position so that the tube is deep enough to prevent any splashes from occurring during a sipper flush, but above the round opening.
 - c Click **Set**, then **Next**.
- 9 When the Set the P-1 Position window appears, proceed according to the following directions, which use the 384-well plate as an example.
 - a Manually adjust the stage so that:
 - the sipper tube goes all the way down into the P-1 well of a 384-well plate, and
 - the sipper tube is properly centered within the well.
 - b Slowly and carefully lower the stage until the sipper tube flexes only slightly, as shown in the photos. Move the stage up a notch from this position and watch the sipper tube bounce back down.



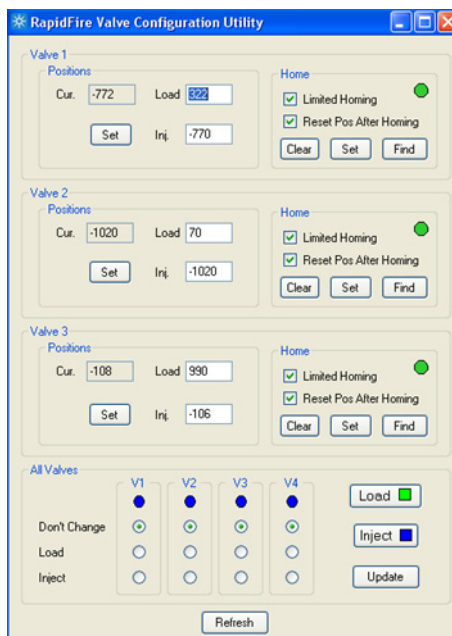
- 10 Repeat [step 9](#) to define positions A-1 and A-24 of the 384-well plate or H1, A1, and A24 of a 96-well plate.
- 11 When the **Set the Matrix 1-4 Position** window appears, specify where you want the sipper tube to travel in the matrix bottles 1-4.
- 12 Click **Swap**, then close the Sipper Configuration Wizard.

1 System Preparation

To check the valves

To check the valves

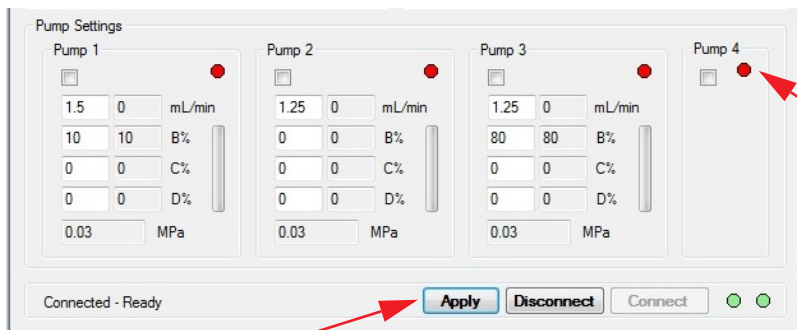
- 1 To open the Valve Configuration Utility dialog box, click **System Tools > Valve Tuner**.



- 2 Check the status of the three nanovalves (Valve 1, Valve 2, and Valve 3) in the lower part of the screen. For normal operation, the status icons are either blue (Inject) or green (Load) for all three valves.
- 3 If the status lights for the valves are yellow or if the Home icons are red, then home the valves by clicking their **Find** buttons.
The Home icons should turn green, and their **Cur. Positions** indicate 0.
- 4 Check that switching the valves from their load to their inject position is smooth and quiet.

To prepare the peristaltic pump

- 1 Turn on the peristaltic pump connected to the wash stations:
 - a Select Pump 4 as **Active**.
 - b Click **Apply**.



- 2 Check that the peristaltic pump is rotating clockwise.
 - You can adjust the flow rate of the peristaltic pump manually by pressing the up and down arrows on its control panel.
 - If the peristaltic has been off for hours, then the organic channel takes a few minutes longer than the aqueous one to reach its chimney steadily.



1 System Preparation

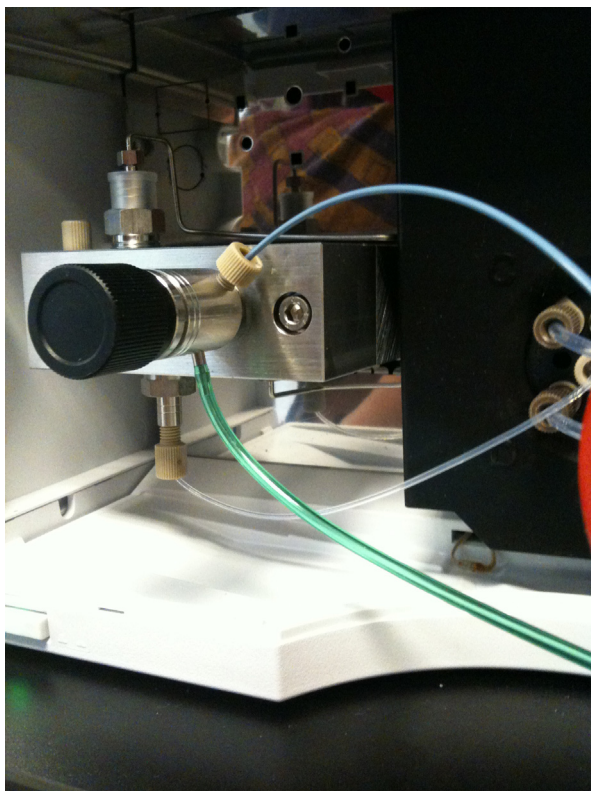
To prepare the quaternary pumps

- 3 With the vacuum on, check that fluid is being aspirated from the drain (central trough) sufficiently. Be sure that no overflow occurs on the wash station.

To prepare the quaternary pumps

Use this procedure to prime the quaternary pump, which removes air bubbles in the fluidic lines. For each pump, prime each one of its four channels, starting with a solvent composition of [100% A, 0% B, 0% C, 0% D] for Pump 1, Pump 2, and Pump 3.

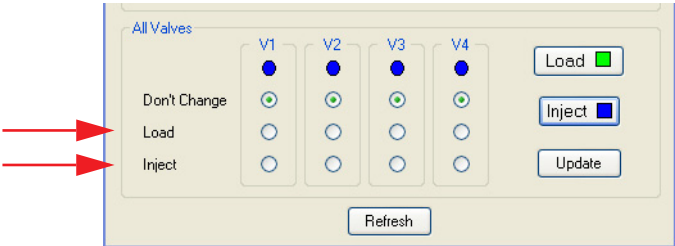
- 1 Turn the diversionary valve (black knob) counterclockwise 90 to 180 degrees to open the valve.



- 2 To turn on the pump, click **Apply** with the pump selected as Active (Pumps 1, 2, and 3).
- 3 Increase the flow rates (step-wise: 3 mL/min, 5 mL/min...) to 10 mL/min, and let the pumps operate for 2 minutes to prime channels A.
To change the flow rate, enter the desired value in mL/min in the Pump Settings panel and click **Apply**.
- 4 Continue to prime the other channels as follows:
 - a Enter a solvent composition of [0% A, 100% B, 0% C, 0% D] for Pump 1, Pump 2, and Pump 3, and click **Apply**. Let the pumps operate at 10 mL/min for 2 minutes to prime channels B.
 - b Enter a solvent composition of [0% A, 0% B, 100% C, 0% D] for Pump 1, Pump 2, and Pump 3, and click **Apply**. Let the pumps operate at 10 mL/min for 2 minutes to prime channels C.
 - c Enter a solvent composition of [0% A, 0% B, 0% C, 100% D] for Pump 1, Pump 2, and Pump 3, and click **Apply**. Let the pumps operate at 10 mL/min for 2 minutes to prime channels D.
- 5 After priming all channels of Pump 1, Pump 2, and Pump 3, set the flows to the assay-specific rates, such as 1.25 mL/min.
- 6 Turn the diversionary valve clockwise to close it. *Tighten it finger-tight only.*
- 7 Check the pressure readings, keeping in mind the following:
 - You can view the pump pressure history on the **System Tools > Pump Pressure Graph** plots.
 - The maximum system pressure is 20 MPa.
 - a To open the Valve Configuration Utility dialog box, click **System Tools > Valve Tuner**.
 - b Select the **Inject** (blue) position for Valves 1, 2, and 3 and check the back pressure of each pump.
 - c Select the **Load** (green) position for Valves 1, 2, and 3 and check the back pressure of each pump.

1 System Preparation

To prepare the quaternary pumps



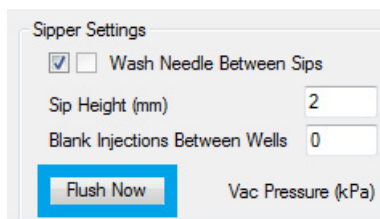
Here are some typical values:

Valve position	Pump number	Back pressure
V1, V2, and V3 blue (Inject position)	P1	~1 - 10 MPa (or 10 - 100 bar)
	P2	~0.1 - 3 MPa
	P3	~1 - 10 MPa
V1, V2, and V3 green (Load position)	P1	~0.1 - 10 MPa (lower than in blue or Inject)
	P2	~0.1 - 3 MPa (higher than in blue or Inject)
	P3	~1 - 10 MPa (higher than in blue or Inject)

If the value is outside the typical range shown for each pump at load or inject position, refer to the *RapidFire 365 High-throughput Mass Spectrometry System Troubleshooting Guide*.

To flush the sipper tube

- 1 Click **Flush Now** in the Sipper Settings area of the UI window.



The following actions take place automatically:

- Pump 1 is turned on.
- The sipper tube is positioned above the wash station drain.
- Valve 4 is actuated to **Load** position.
- The flow rate for Pump 1 is ramped up to 8 mL/min. This rate is maintained for 1 minute, then is reduced to its default flow rate setting.
- Pump 1 is turned off.
- Valve 4 is rotated back to **Inject** position.
- The stages are moved to the **Swap** position.

1 System Preparation

To prepare the Agilent MassHunter Q-TOF LC/MS computer

To prepare the Agilent MassHunter Q-TOF LC/MS computer

Before you begin Confirm that Agilent Data Acquisition software is installed on the computer.

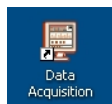
- 1 Click **RapidFire Communicator** on the Windows Start menu or desktop.
The following program files are started:

- **RFRemServer.exe**
- **RFMassunterS_TOF.exe**

These program files are required for the software integration of the RapidFire System.

Image Name	User Name	CPU	Mem Usage
lsass.exe	SYSTEM	00	1,324 K
nsvsc32.exe	SYSTEM	00	4,032 K
RFMassHunterS_TOF.exe	HTMS-team	00	24,288 K
RFRemServer.exe	HTMS-team	00	20,080 K

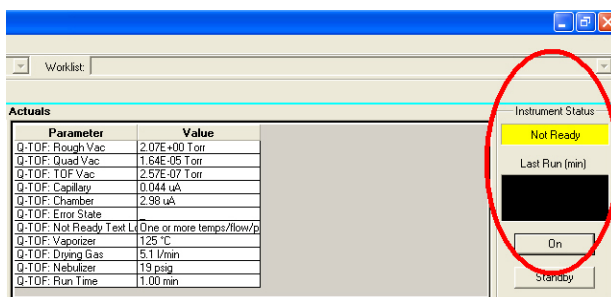
- 2 To start the MassHunter Data Acquisition software, double-click the **Data Acquisition** desktop icon.



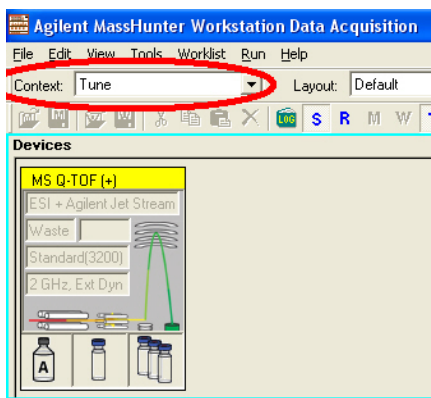
- 3 Turn on the detector.

The Instrument Status box turns from yellow (Not Ready) to green (Ready).

To prepare the Agilent MassHunter Q-TOF LC/MS computer



4 Select **Tune** in the Context list box, as shown in the following figure.



5 Set the mass and resolution parameters for your analysis in the Instrument State tab of the bottom panel.

Tip

- High Resolution (scan speed 4 GHz)
- Extended Dynamic Range (scan speed 2 GHz)

1 System Preparation

To prepare the Agilent MassHunter Q-TOF LC/MS computer

- Minimum Storage Size (scan speed 1 GHz)

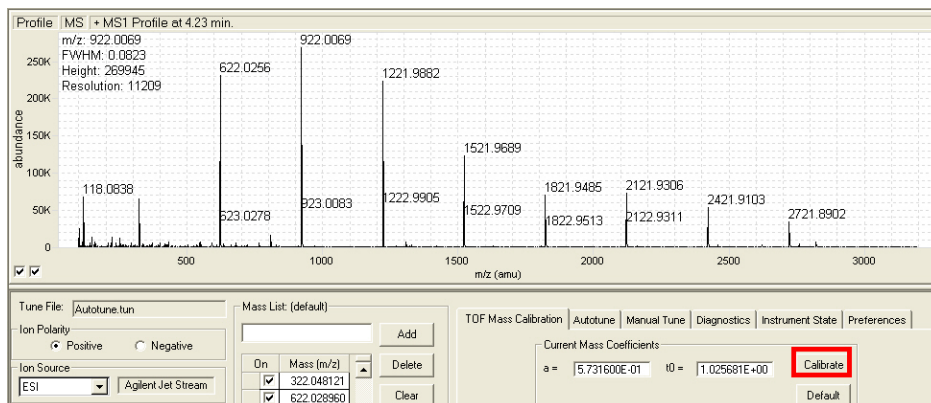
a Click **Apply** on the right.

The screenshot shows the 'Autotune' tab in the Agilent MassHunter software. The 'Tune File' is 'Autotune.tun'. The 'Mass List' contains several peaks. The 'Calibrant Bottle' is set to 'None'. The 'Collision Energy' is set to '0'. The 'Fast Polarity Switching' is set to 'Low (1700 m/z)'. The 'Instrument Mode' is set to 'Extended Dynamic Range'. The 'Apply' button is highlighted with a red box.

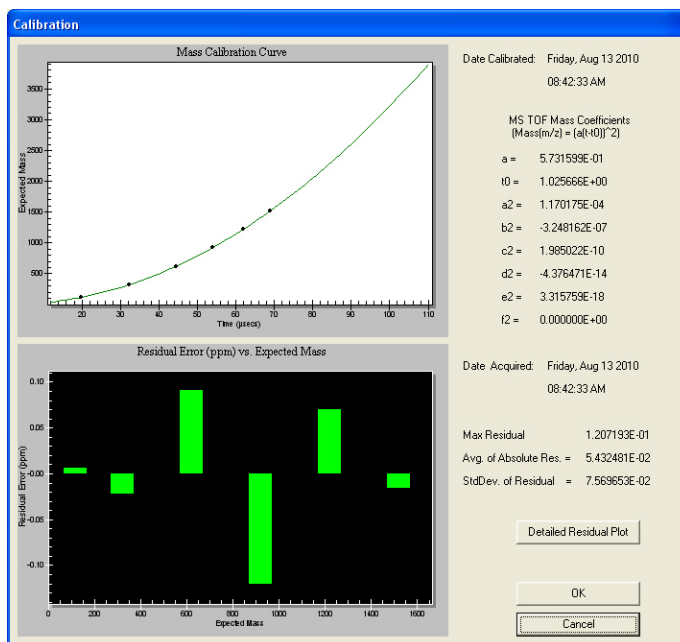
6 Calibrate the mass spectrometer.

a Set the location of the Calibrant Bottle (example: B), and wait for the mass-over-charge spectrum to stabilize.

b Click **Calibrate**.



c When the Calibration results window appears, check that the calculated residual errors are satisfactory (for example < 2.0 ppm).



d Click **OK** to close the results window.

e Set the Calibrant Bottle flag back to **None**.

7 Select **Acquisition** in the Context list box.

If prompted, confirm that you want to operate under the modified Instrument State. **DO NOT modify the Tune File.**

8 Click **File > Open > Method** to open a .m method or set parameters to create a method.

Methods for the RapidFire System are stored in the **D:\MassHunter\ methods\ RapidFire** folder.

9 Set the parameters for your analysis on the **MS Q-TOF** tabs shown in the following example screens.

1 System Preparation

To prepare the Agilent MassHunter Q-TOF LC/MS computer

Sample | Properties | **MS Q-TOF**

Ion Source: ESI Ion Polarity: Positive Data Storage: Profile LC Stream: MS

Agilent Jet Stream

Stop Time: ☒ No Limit/As Pump ☐ Stop Time min

Cycle Time: s

Time Segment and Experiment #

Time (min)	Expt #
0	1

General | Source | Acquisition | Ref Mass | Chromatogram

Ion Polarity (Seg): ☒ Positive ☐ Negative ☐ Fast Polarity Switching

Data Storage (Seg): ☐ None ☐ Centroid ☐ Both ☒ Profile

Do not wait for setpoints (e.g. temperature) to equilibrate

LC Stream (Seg): ☒ MS ☐ Waste

Plot and Centroid Data Storage Threshold

MS		MS/MS	
Abs. threshold	200	Abs. threshold	5
Rel. threshold (%)	0.01	Rel. threshold (%)	0.01

Sample | Properties | **MS Q-TOF**

Ion Source: ESI Ion Polarity: Positive Data Storage: Profile LC Stream: MS

Agilent Jet Stream

Stop Time: ☒ No Limit/As Pump ☐ Stop Time min

Cycle Time: s

Time Segment and Experiment #

Time (min)	Expt #
0	1

General | Source | Acquisition | Ref Mass | Chromatogram

ESI (Seg): Gas Temp °C °C

Drying Gas l/min l/min

Nebulizer psig psig

Sheath Gas Temp °C °C

Sheath Gas Flow l/min l/min

MS TOF (Expt): Fragmentor V

Skimmer V

OCT 1 RF Vpp V

ESI (Expt): VCap V Capillary uA

Chamber uA

Nozzle Voltage (Expt) V

Sample | Properties | **MS Q-TOF**

Ion Source: ESI Ion Polarity: Positive Data Storage: Profile LC Stream: MS

Agilent Jet Stream

Stop Time: ☒ No Limit/As Pump ☐ Stop Time min

Cycle Time: s

Time Segment and Experiment #

Time (min)	Expt #
0	1

General | Source | Acquisition | Ref Mass | Chromatogram

Mode: ☒ MS (Seg) ☐ Auto MS/MS (Seg) ☐ Targeted MS/MS (Seg)

TOF Spectra

Mass Range: Min Range m/z Max Range m/z

Acquisition Rate/Time: Rate spectra/s Time ms/spectrum Transients/spectrum

To prepare the Agilent MassHunter Q-TOF LC/MS computer

Sample | Properties | **MS Q-TOF** | Data Storage | LC Stream | General | Source | Acquisition | Ref Mass | Chromatogram

Ion Source: ESI | Ion Polarity: Positive | Data Storage: Profile | LC Stream: MS

Agilent Jet Stream

Stop Time: ☐ No Limit/As Pump ☐ Stop Time min

Cycle Time: s

Time Segment and Experiment #

Time (min)	Expt #
0	1

Reference Mass Correction

☐ Enable

☒ Use bottle A

Reference Masses

On	M/Z
☐	121.0509
☒	922.0098
☒	149.02332

Auto Recalibration Parameters

Average: scans

Reference Mass

Detection Window: ppm

Minimum Height: counts

Sample | Properties | **MS Q-TOF** | Data Storage | LC Stream | General | Source | Acquisition | Ref Mass | **Chromatogram**

Ion Source: ESI | Ion Polarity: Positive | Data Storage: Profile | LC Stream: MS

Agilent Jet Stream

Stop Time: ☐ No Limit/As Pump ☐ Stop Time min

Cycle Time: s

Time Segment and Experiment #

Time (min)	Expt #
0	1

Chromatograms

Chromatogram	Label	Expt Type	Polarity Type	Offset	Y-Range
TIC	TIC	MS	Both	15	10000000

10 When you have finished setting the parameters, click **Apply** on the right side of the screen.

Properties | **MS Q-TOF** | Data Storage | LC Stream | General | Source | Acquisition | Ref Mass | **Chromatogram**

Ion Source: ESI | Ion Polarity: Positive | Data Storage: Profile | LC Stream: MS

Agilent Jet Stream

Stop Time: ☐ No Limit/As Pump ☐ Stop Time min

Cycle Time: s

Time Segment and Experiment #

Time (min)	Expt #
0	1

Chromatograms

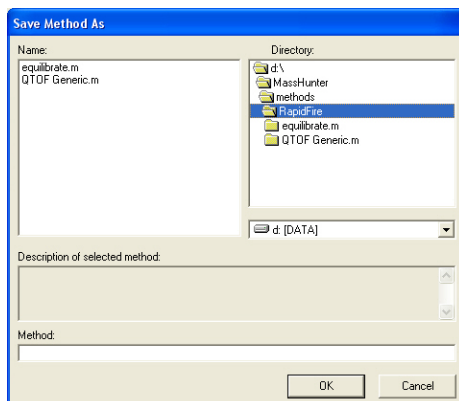
Chromatogram	Label	Expt Type	Polarity Type	Offset	Y-Range
TIC	TIC	MS	Both	15	10000000

1 System Preparation

To prepare the Agilent MassHunter Q-TOF LC/MS computer

11 Save the method.

Save all **.m** methods for use with RapidFire System in the **D:\MassHunter\methods\ RapidFire** folder.



To prepare the Agilent MassHunter Triple Quad MS computer

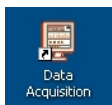
Before you begin Confirm that MassHunter Data Acquisition software is installed on the computer.

- 1 Click **RapidFire Communicator** on the Windows Start menu or desktop.
The following program files are started:

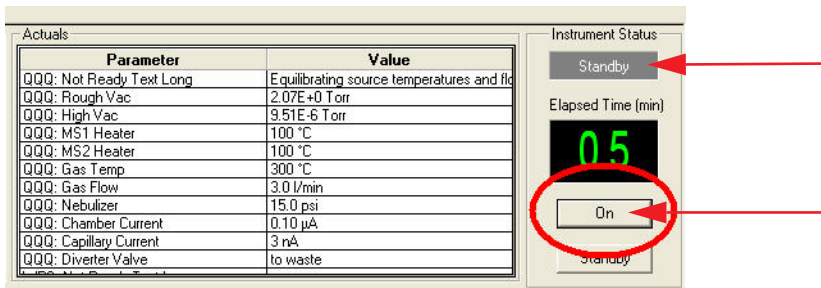
- **RFRemServer.exe**
- **RFMassunterS_QqQ.exe**

These program files are required for the software integration of the RapidFire System.

- Tip** You can verify that the programs are running using Windows Task Manager.
- 2 To start the MassHunter Data Acquisition software, double-click the **Data Acquisition** desktop icon.



- 3 To turn on the detector, click **On**.



The Instrument Status box turns from yellow (Not Ready) to green (Ready).

1 System Preparation

To prepare the ABI/Sciex Triple Quad MS computer

To prepare the ABI/Sciex Triple Quad MS computer

Before you begin Confirm that an appropriate version of Analyst software is installed on the computer.

- 1 Click **RapidFire Communicator** on the Windows Start menu or desktop.
The following program files are started:

- **RFRemServer.exe**
- **RFAAOS.exe**

These program files are required for the software integration of the RapidFire System.

Tip You can verify that the program files are running using Windows Task Manager.

To turn off the system at the end of the day

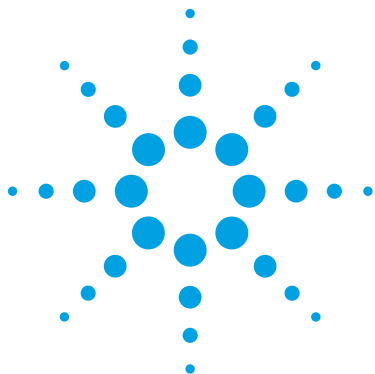
Do these steps when you are done using the RapidFire System for the day.

NOTE

The instrument goes into stand-by mode at the end of each series of plates. This precaution ensures that all pumps are turned off at the end of a run.

- 1 If your samples are a high-salt high-protein or buffers, then:
 - a Flush the sipper to avoid the formation of precipitates as described in [“To flush the sipper tube”](#) on page 23.
 - b Clean the fluidics lines by running a few rows of methanol wells. A minimum of 50 wells is recommended.
- 1 Close the RapidFire Control software.
 - a In the RapidFire window, click **Disconnect** to disconnect the software from the RapidFire instrument.
 - b Close the RapidFire window.
 - c In the RapidFire Control Panel, click **Stop**.
 - d Close the RapidFire Control Panel.
- 2 After idling for about 20 minutes, most mass spectrometers will automatically go into stand-by mode. If that is not the case of your system, actively put it into stand-by mode by following the instructions in your mass spectrometer documentation.
- 3 Turn off the vacuum source.

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2 Maintenance

- To prepare the instrument for daily use 36
- To maintain the system at the end of each day 36
- To maintain the system at the end of the week 36
- To shut down the RapidFire System 37
- To resume operation after a power shutdown 37

This chapter contains procedures to help you maintain the RapidFire System.



2 Maintenance

To prepare the instrument for daily use

To prepare the instrument for daily use

- 1 Replace solvents.
- 2 Change the cartridge.
- 3 Home the stages.
- 4 Check the status of the valves.
- 5 Prime or flush all pumps lines.
- 6 Flush the sipper tube.

To maintain the system at the end of each day

- 1 Flush the sipper tube again as described in “To flush the sipper tube” on page 23.
- 2 Do the shutdown procedures described in “To turn off the system at the end of the day” on page 33.

To maintain the system at the end of the week

- 1 Empty the waste containers (carboy and vacuum flask).
- 2 Clean the mass spectrometer according to the instructions provided by the manufacturer.

To shut down the RapidFire System

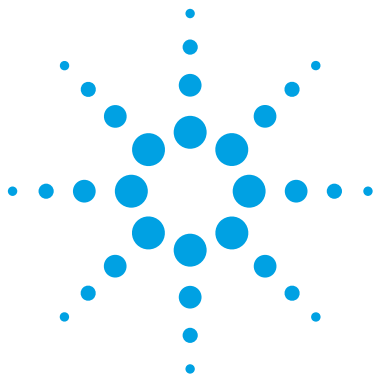
Do these steps to turn off the RapidFire System completely.

- 1 Close the RapidFire Control software.
 - a In the RapidFire window, click **Disconnect** to disconnect the software from the RapidFire instrument.
 - b Close the RapidFire window.
 - c In the RapidFire Control Panel, click **Stop**.
 - d Close the RapidFire Control Panel.
- 2 Safely turn off the RapidFire computer.
- 3 Turn off the pumps and the plate handler.
- 4 Turn off the UPS unit.

To resume operation after a power shutdown

- 1 Turn on the UPS unit.
- 2 Turn on the pumps and the plate handler.
- 3 Turn on the RapidFire computer.
- 4 Start the RapidFire Control Software. See [“To start the software”](#) on page 11.

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3 Operation in Plates Mode

- To prepare plates 40
- To load plates manually 40
- To load plates using the BenchBot plate handler 41
- To prepare the Agilent MassHunter Q-TOF LC/MS computer 42
- To prepare the Agilent MassHunter Triple Quad LC/MS computer 43
- To prepare the ABI/Sciex Triple Quad MS computer 45
- To prepare the Thermo Fisher Triple Quad MS computer 47
- To set RapidFire run parameters 49
- To start a run in Plates mode 52
- To pause a run 53
- To stop a run 53
- To make an emergency stop 54
- To maintain a log book of data 55

This chapter contains procedures to help you operate the RapidFire 365 High-throughput Mass Spectrometry System in Plates Mode.



3 Operation in Plates Mode

To prepare plates

To prepare plates

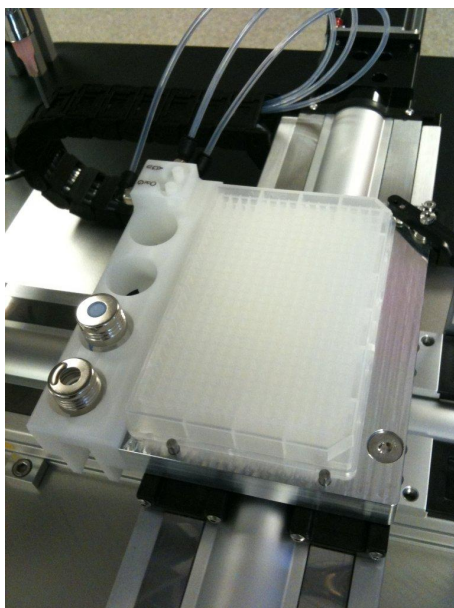
- 1 Completely thaw the sample plates.
- 2 Centrifuge the plates at 3000 rpm for at least 5 minutes.

Tip Spin down the plates, particularly if samples contain insoluble particulates, such as microsomes. When you spin the plates, particles and insoluble material are forced to the bottom of the wells where they are less likely to be aspirated into the system.

To load plates manually

You can either load plates manually as described here or use the BenchBot plate handler as described in the next topic.

- 1 Check that the sipper has been homed as described in [“To home the stages”](#) on page 14.
- 2 Securely position the plate on the holder in between the metal pins, then close the front doors.



To load plates using the BenchBot plate handler

You can either load plates using the BenchBot plate handler as described here or manually as described in the previous topic.

- 1 On the main RapidFire System window:
 - a Select the plate configuration (96- or 384-well).
 - b Mark the **Use Plate Handler** option. Click **Apply** to register the change.

The screenshot shows the 'Plate Info' and 'Miscellaneous Settings' sections of the RapidFire System configuration window. In the 'Plate Info' section, the 'Plate Configuration' dropdown menu is set to 'StandardPlate_96', with a red arrow pointing to it. In the 'Miscellaneous Settings' section, the 'Use Plate Handler' checkbox is checked, also indicated by a red arrow. Other settings include 'Plates Completed' at 0, 'Input Stack' and 'Output Stack' at NA, 'Current Column Position' at Unknown, 'Use Barcode Scanner' unchecked, 'Mass Spec Standby After Run' checked, 'Plates Between Flushes' at 1 and 4, and 'Missed Sip Tolerance' at 5 and 10.

Plate Info	
Current Plate Name	
Plates Completed	0
Input Stack	NA
Output Stack	NA
Current Column Position	Unknown
Plate Configuration	StandardPlate_96

Miscellaneous Settings	
☒ ☒ Use Plate Handler	
☐ ☐ Use Barcode Scanner	
☒ ☒ Mass Spec Standby After Run	
Plates Between Flushes	1 4
Missed Sip Tolerance	5 10

- 2 Stack the plates to be analyzed in the input hotels.

3 Operation in Plates Mode

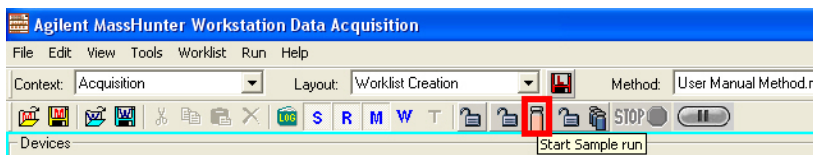
To prepare the Agilent MassHunter Q-TOF LC/MS computer

To prepare the Agilent MassHunter Q-TOF LC/MS computer

- 1 Set up the mass spectrometer computer and turn on the mass spectrometer as described in “To prepare the Agilent MassHunter Q-TOF LC/MS computer” on page 24.
- 2 On the **Sample Tab** of the Method Editor pane in the MassHunter Data Acquisition software:
 - a Enter the **Name** and **Path** for the data file.
The data file Name must be **sequence1.d** (for data analysis by RapidFire Integrator).
 - b Set the Run Type to **Manual Start**.
 - c Set the Injection Volume to **-1 µL**. This value causes the system to use the injection volume set by the RapidFire injection loop (at V1P2-V1P5).

The screenshot shows the 'Sample' tab configuration in the MassHunter Data Acquisition software. The 'Name' field is set to 'User Manual Sample' and the 'Position' is 'Vial 1'. The 'Data File' section has 'Auto-Increment' checked, with 'Name' set to 'sequence1.d' and 'Path' set to 'D:\MassHunter\Data\RapidFire\User Guide\'. The 'Run' section shows 'Run Type' as 'Manual Start', 'Part of method to run' as 'Acquisition Only', 'Operator Name' as an empty field, 'Wait Time for Ready' as '10 (min)', and 'Injection Volume' as '-1 (µl)'. A green oval highlights the 'Data File' section.

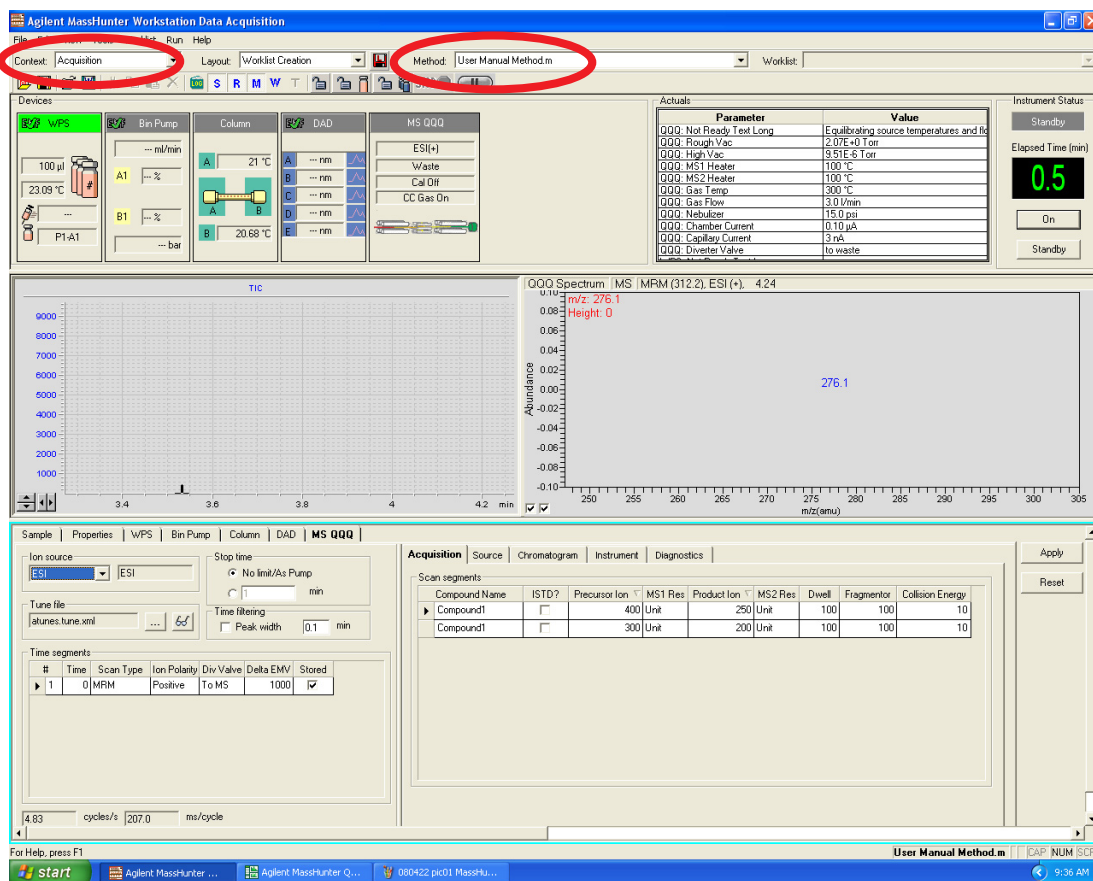
- 3 Click the **Start Sample** button.



When the source has equilibrated, the label of the MS Q-TOF device module turns blue in the Instrument Status pane.

To prepare the Agilent MassHunter Triple Quad LC/MS computer

- 1 Set up the mass spectrometer computer and turn on the mass spectrometer as described in “To prepare the Agilent MassHunter Triple Quad MS computer” on page 31.
- 2 Select **Acquisition** in the Context list box.



- 3 To open a .m method or set parameters to create a method, click **File > Open > Method**.

Agilent suggests storing methods used for the RapidFire-MS System in the **D:\MassHunter\ methods\ RapidFire** folder.

3 Operation in Plates Mode

To prepare the Agilent MassHunter Triple Quad LC/MS computer

- 4 Set the parameters for your analysis on the **MS QQQ** tabs.
- 5 On the **Sample Tab** of the Method Editor pane in the MassHunter Data Acquisition software:
 - a Enter the **Name** and **Path** for the data file.
 - The data file **Name** must be **sequence1.d** (for data analysis by RapidFire Integrator).
 - A commonly used **Path** setting is **D:\MassHunter\ Data\ RapidFire**.
 - b Set the Run Type to **Manual Start**.
 - c Set the Injection Volume to **-1** μL . This value causes the system to use the injection volume set by the RapidFire injection loop (at V1P2-V1P5).

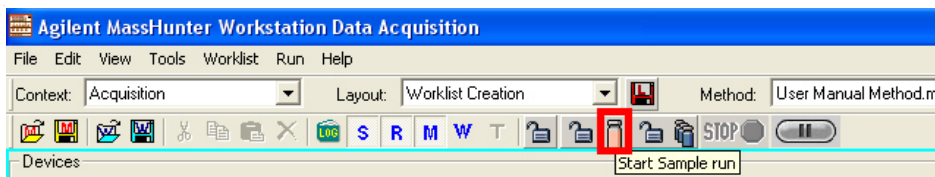
Sample
Name: User Manual Sample Position: Vial 1

Custom1: Custom2:

Data File
☒ Auto-Increment
Name: sequence1.d
Path: D:\MassHunter\Data\RapidFire\User Guide\.. View Data

Run
Run Type: Manual Start
Part of method to run: Acquisition Only
Operator Name:
Wait Time for Ready: 10 (min)
Injection Volume: -1 (μl)
Comment:

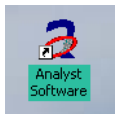
- 6 Click the **Start Sample** button.



When the source has equilibrated, the label of the MS QQQ device module turns blue in the Instrument Status pane.

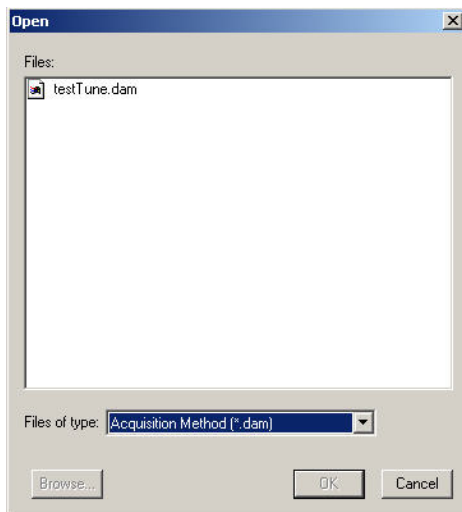
To prepare the ABI/Sciex Triple Quad MS computer

- 1 Set up the mass spectrometer computer and turn on the mass spectrometer as described in “To prepare the ABI/Sciex Triple Quad MS computer” on page 32.
- 2 On the MS computer, launch Analyst by double-clicking its icon on the desktop.



- 3 In Tune mode, load appropriate project and acquisition method files.
- 4 Click **Tools > Create subproject**, and save this method in a subproject folder with the current date.

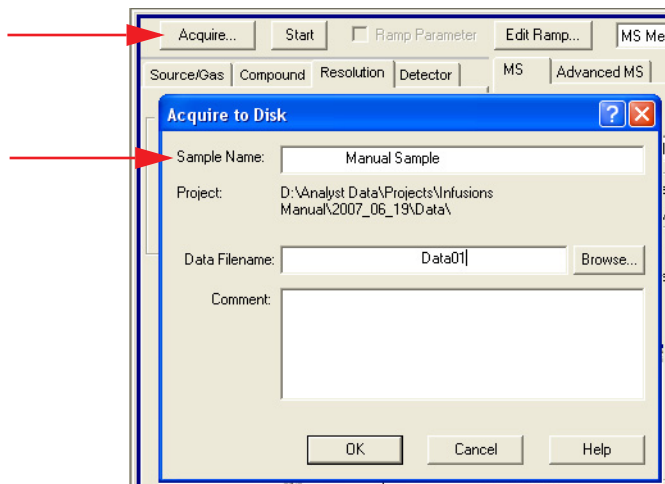
In the following example, project= **Infusions Manual**, subproject=**2007_06_19**, and method= **testTune.dam**.



3 Operation in Plates Mode

To prepare the ABI/Sciex Triple Quad MS computer

- 5 Click **Acquire** and type in a name for your sample.

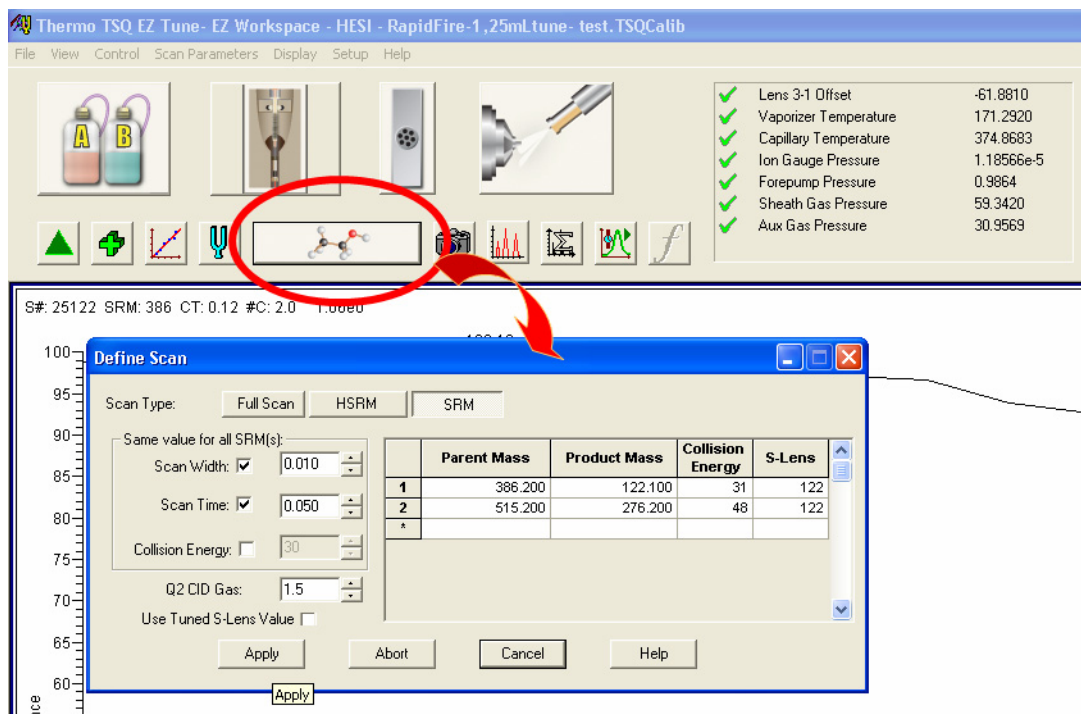


The data will be recorded in a file named **<DataFileName.wiff>** in the **Project / Subproject** folder.

- 6 Click **OK** to start the run.

To prepare the Thermo Fisher Triple Quad MS computer

- 1 Start Xcalibur.
- 2 Load the appropriate method file using the **Define Scan** window.

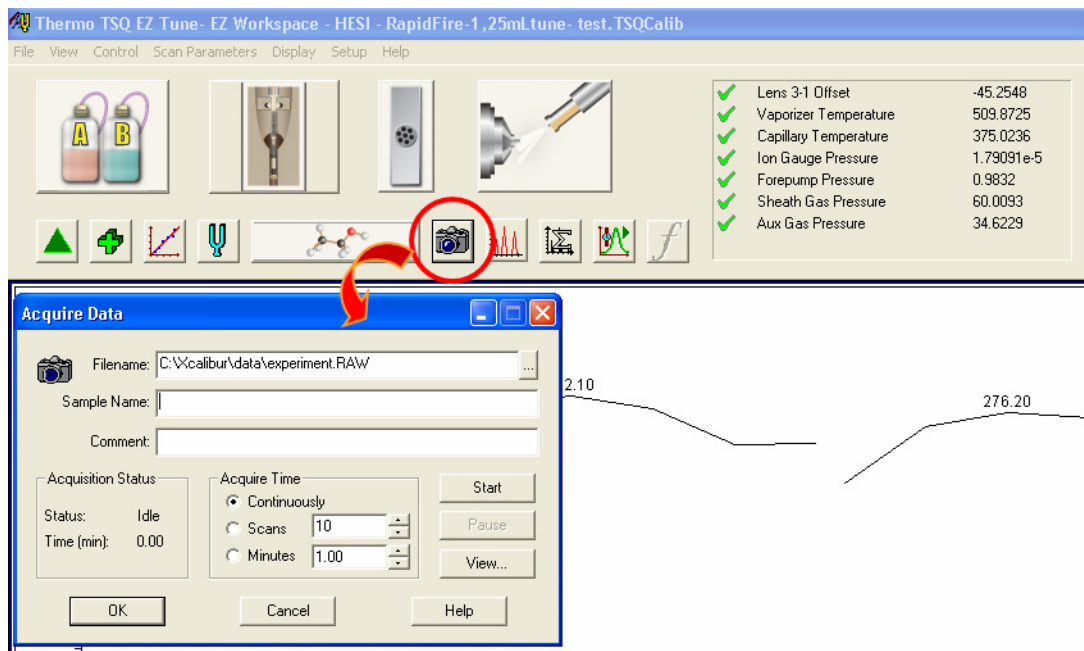


- 3 Click **Apply** to take these new parameters into consideration.
Confirm that you are using the **RapidFire-1,25mL tune.test** settings.
- 4 Wait until all source settings such as temperatures and flows have stabilized.
- 5 Select the folder where you want to save the data as follows:
 - a Click the camera button.
 - b Browse through your data file tree.
 - c Select the appropriate file name.

3 Operation in Plates Mode

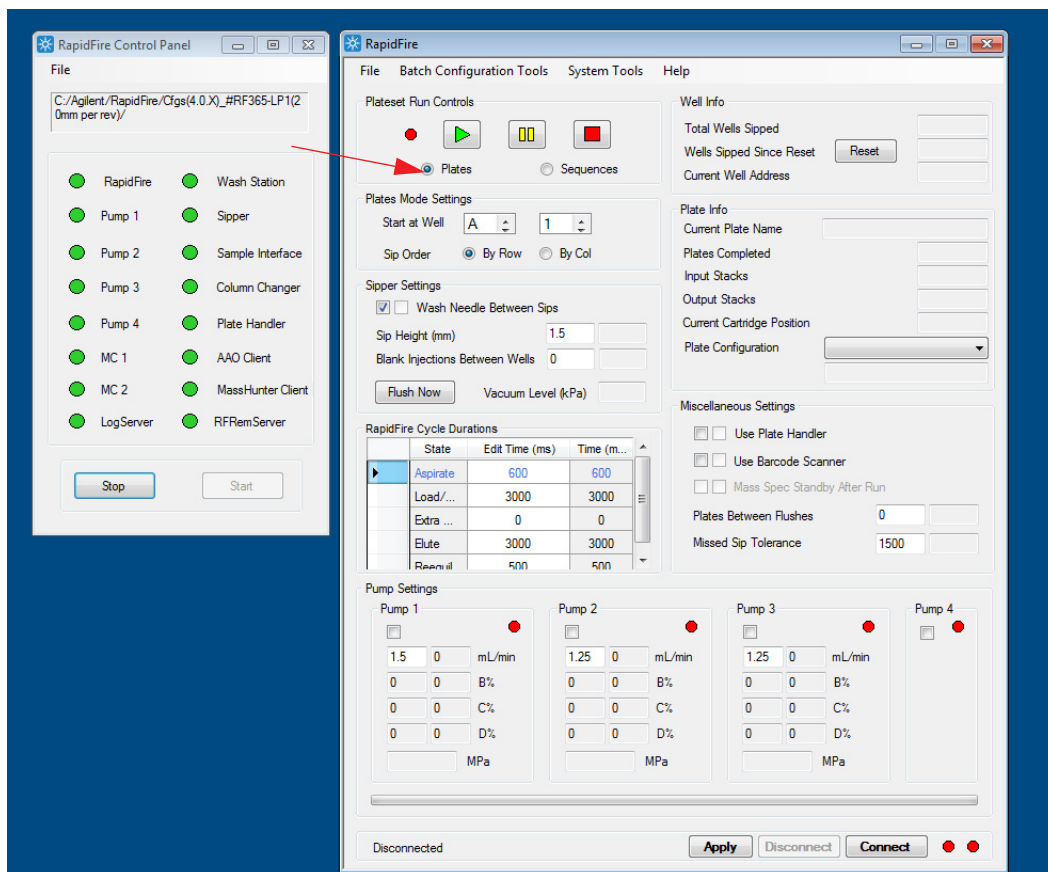
To prepare the Thermo Fisher Triple Quad MS computer

6 Click the **Start** button to begin data acquisition and recording.



To set RapidFire run parameters

- 1 Click **System Tools > Cartridge Changer**.
- 2 When the Cartridge Changer dialog box appears:
 - a Open the column select list and select the solid-phase extraction (SPE) cartridge appropriate for your assay.
 - b Click **Go To**.
 - c Close the Cartridge Changer dialog box.
- 3 Set the parameters for your analysis on the RapidFire software.
 - Be sure to select the **Plates** option.



3 Operation in Plates Mode

To set RapidFire run parameters

- 4 In the **Plates Mode Settings** area:
 - a In the **Start at Well** field, type the position on the plate to start the run. The first position is **A1**.
 - b Select to read microtiter plates in *either* of the following ways:
 - **By Row:** A1, A2, A3, ..., H11, H12,
 - **By Column:** A1, B1, C1, ..., G12, H12.
- 5 In the **Sipper Settings** area of the screen:
 - a Mark the **Wash Needle Between Sips** option to wash the sipper tube once after aspiration of the sample from each well. The tube is washed first in the aqueous, then in the organic wash chimney, for 300 ms in each.
 - b In the **Sip Height** field, type the number of millimeters from the *lowest position* of the sipper tube to the bottom of the well. Enter a value of 1.0 mm or greater. The sipper tube (in V1P3) travels down from Safe Z into the sample well until *either*:
 - The sip sensor (in V1P4) optically detects the presence of fluid and triggers the actuation of V1, which causes the transition between State # 1 (Aspiration) and State # 2 (Load/Wash), *or*
 - The z-position of the sipper tube reaches the set **Sip Height**.
 - c Enter a value > 0 for **Blank Injections Between Wells** to have the system do extra cycles in the organic wash station *between wells*. This setting helps to limit or prevent carryover between samples.
- 6 In the **RapidFire Cycle Durations** area, set the valve timing, as per assay protocol for States 1 - 5 (**Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, and **Re-equilibrate**).
 - Set the value of **Aspirate** to at least 600 ms for reliable sip sensor triggering.
- 7 In the **Well Info** area, you can view the following information:
 - the number of wells sipped in the lifetime of your RapidFire System
 - the number of wells sipped since the last reset
 - the location of the Current Well Address (such as [1,7] for position A7).
- 8 In the **Plate Info** area, select either **96 Well** or **384 Well** as the Plate Configuration.

- 9 In the **Miscellaneous Settings** area:
 - a Mark whether to:
 - **Use Plate Handler**
 - **Use Barcode Scanner**
 - Put the **Mass Spec in Standby After Run**.
In Plates mode, the option to communicate with the mass spectrometer is not available.
 - b Set the **Plates Between Flushes** as follows:
 - For 96-well plates, use **4**.
The sipper tube flushes for 1 minute after *every 4 plates*.
 - For 384-well plates, use **1**.
The sipper tube flushes for 1 minute after *every plate*.
 - c Set the **Missed Sips Tolerance** to a value > 0 to suspend RapidFire System operation after the specified number of wells are found to be empty by the sip sensor.
- 10 In the **Pump Settings** area at the bottom of the screen, set pump flow rates and solvent compositions for your assay.
- 11 Click **Apply**.
- 12 To save the parameter settings made in the previous steps for future use, click **File > Save RF Method As**.

To start a run in Plates mode

- 1 Click the **Play** button in the upper left area of the screen: 
- 2 When prompted in a command window, enter the plate name with the keyboard or using the hand-held barcode scanner to input the barcode, then click **OK**.
- 3 Click the **Play** button again.
- 4 Verify that the sipper tube aspirated the first few samples.
- 5 Verify that the analytes are being detected by the mass spectrometer.
Runs in Plates mode are *not* synchronized with the mass spectrometer. Initiate the mass spectrometer acquisition run *before* RapidFire starts.

NOTE

Close the doors while operating the RapidFire System to prevent possible injury. The system does not operate with the doors open.

- 6 If you are loading plates manually, then when the plate run is completed, you are prompted to load a new plate.
 - a If you have more plates to read, then:
 - Remove the previous plate.
 - Position your next plate on the deck as described in “[To load plates manually](#)” on page 40.
 - Enter a name for the new plate using the keyboard or the hand-held barcode scanner, then click **OK**.
If you do not enter a plate name within 30 minutes, then a time-out feature automatically puts the RapidFire System in stand-by mode to keep solvent from being wasted.
 - b If all plates in this set have been run, then click **Cancel**.

To pause a run

Use this procedure to suspend a run temporarily.

- 1 Click the **Pause** button .

- The cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, **Re-equilibrate**, and **Needle Wash** is completed for the last sample injected, then the RapidFire System pauses operation.
- The mass spectrometer continues to collect “baseline” data.
- The RapidFire System pumps continue to run.

- 2 To resume operation after pausing, click the **Play** button .

- The system starts up where it left off and data is saved to the current data file.
- The remaining samples are run in the original order.
- If the doors are left open, the system does not resume operation.

To stop a run

- 1 Click the **Stop** button .

- The cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, **Re-equilibrate**, and **Needle Wash** is completed for the last sample injected, then the RapidFire System stops operation.
- Data acquisition is stopped and the current data file is closed.

3 Operation in Plates Mode

To make an emergency stop

To make an emergency stop

CAUTION

Use the E-STOP procedure only during an emergency.

Otherwise, use either of these procedures to stop the RapidFire System:

[“To pause a run”](#) on page 53, or

[“To stop a run”](#) on page 53.

- 1 Press the physical emergency stop button (labeled **E-STOP**), which cuts power to the entire system.



Opening an interlock door of the RapidFire System also stops motion.

- 2 To restart the system, pull up the **E-STOP** button to re-establish power.

To maintain a log book of data

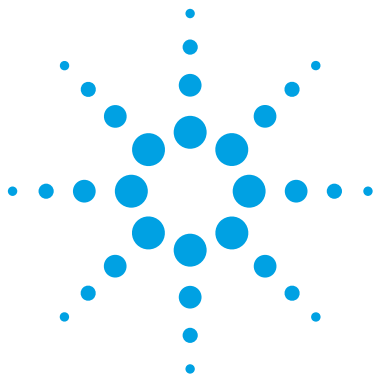
Use a log book to record information about the two sets of data that are stored in two independent systems (the mass spectrometer computer and the RapidFire System computer) in Plates Mode. This record makes it easier to retrieve data and match corresponding data sets in Data Analysis.

Consider including the following information in the logbook, such as in a spreadsheet format:

- **Date**
Generated data is stored by date in the RapidFire System computer. See the *Data Analysis Guide* for more information.
- **Assay description**
To relate to MS methods.
- **Sample name**
To relate to the data file from the MS.
- **Set of plates number** as assigned by RapidFire System.
See the *Data Analysis Guide* for more information.
- **Plate number** (in the current set of plates)
- **Comments**

See Also See the *G9530-90001 RapidFire365 Analysis Guide* for more information.

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4 Operation in Sequences Mode

- To prepare plates 58
- To load plates manually 58
- To load plates using the BenchBot plate handler 59
- To run plates 61
- To prepare the mass spectrometer computer 62
- To set parameters in the RapidFire method 64
- To edit the plate map 66
- To edit the batch 67
- To run a batch in Sequences mode 70
- To pause a run 75
- To stop a run 75
- To make an emergency stop 76

This chapter contains procedures to help you operate the RapidFire 365 High-throughput Mass Spectrometry System in Sequences Mode.



4 Operation in Sequences Mode

To prepare plates

To prepare plates

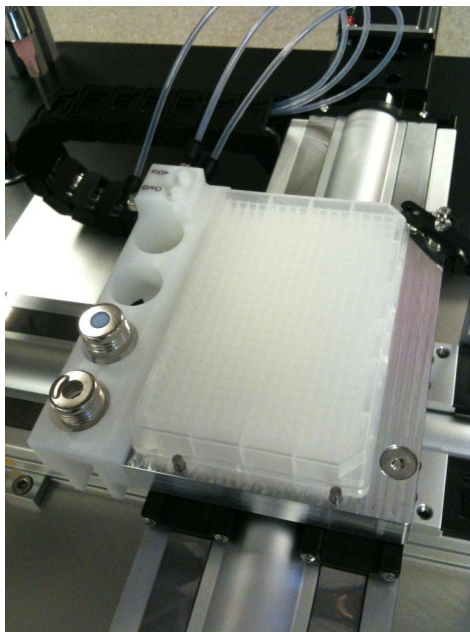
- 1 Completely thaw the sample plates.
- 2 Centrifuge the plates at 3000 rpm for at least 5 minutes.

Tip Spin down the plates, particularly if samples contain insoluble particulates, such as microsomes. When you spin the plates, particulates are forced to the bottom of the wells, where it is less likely to be aspirated into the system.

To load plates manually

You can either load plates manually as described here or using the BenchBot plate handler as described in the next topic.

- 1 Check that the sipper has been homed as described in [“To home the stages”](#) on page 14.
- 2 Securely position the plate on the holder in between the metal pins, then close the front doors.



To load plates using the BenchBot plate handler

You can either load plates using the BenchBot plate handler as described here or manually as described in the previous topic.

- 1 On the main RapidFire System window:
 - a Select the plate configuration (96- or 384-well).
 - b Mark the **Use Plate Handler** option, then click **Apply**.

The screenshot shows the 'Plate Info' and 'Miscellaneous Settings' sections of the RapidFire System configuration window. In the 'Plate Info' section, the 'Plate Configuration' dropdown menu is set to 'StandardPlate_96', with a red arrow pointing to it. In the 'Miscellaneous Settings' section, the 'Use Plate Handler' checkbox is checked, also indicated by a red arrow. Other settings include 'Plates Completed' at 0, 'Input Stack' and 'Output Stack' at NA, 'Current Column Position' at Unknown, 'Use Barcode Scanner' unchecked, 'Mass Spec Standby After Run' checked, 'Plates Between Flushes' at 1 and 4, and 'Missed Sip Tolerance' at 5 and 10.

Plate Info	
Current Plate Name	
Plates Completed	0
Input Stack	NA
Output Stack	NA
Current Column Position	Unknown
Plate Configuration	StandardPlate_96

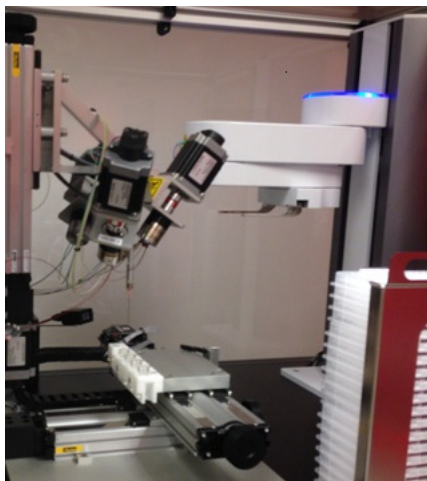
Miscellaneous Settings	
☒ ☒ Use Plate Handler	
☐ ☐ Use Barcode Scanner	
☒ ☒ Mass Spec Standby After Run	
Plates Between Flushes	1 4
Missed Sip Tolerance	5 10

- 2 Stack the plates to be analyzed in the input hotels, as shown in the next figure.

All plates read within a single run must be of the same format. For example, you cannot mix deep-well with regular-height plates within a single run.

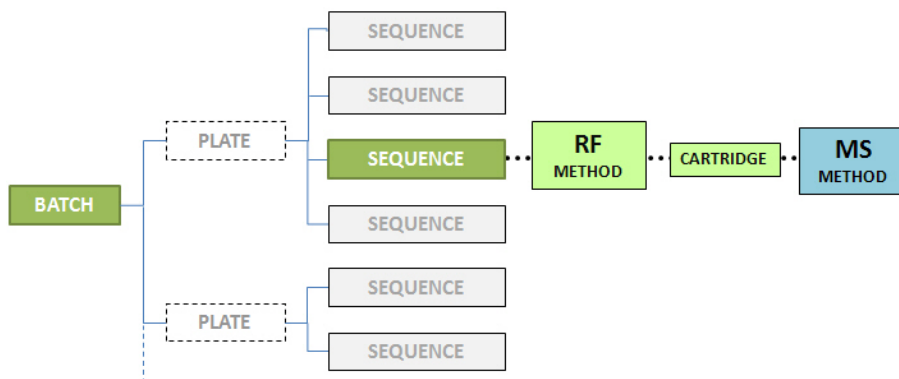
4 Operation in Sequences Mode

To load plates using the BenchBot plate handler



To run plates

The following diagram illustrates the terminology used to define a batch of plates in Sequences mode.



4 Operation in Sequences Mode

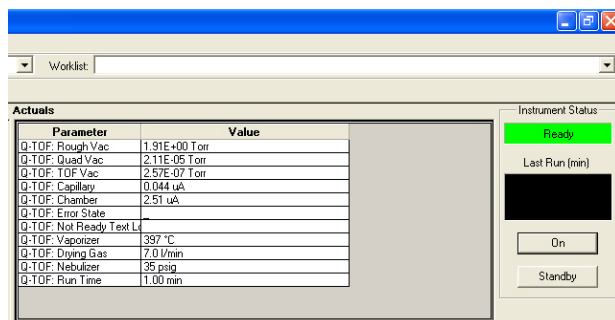
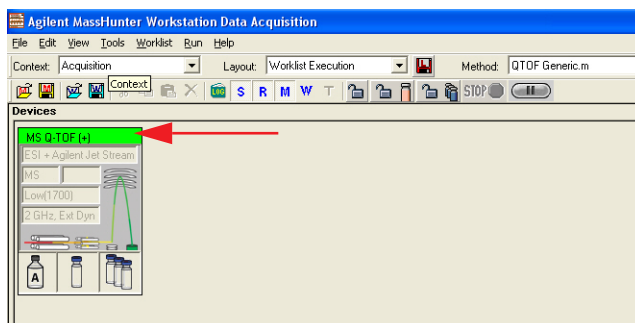
To prepare the mass spectrometer computer

To prepare the mass spectrometer computer

Follow instructions for the appropriate MS system from the “System Preparation” chapter:

- “To prepare the Agilent MassHunter Q-TOF LC/MS computer” on page 24.

Check that the Instrument Status is Ready (green) in the MassHunter Data Acquisition software.



In Sequences mode, with synchronization between the RapidFire System and the MS, data is recorded automatically in **D:\MassHunter\Data\RapidFire**. Refer to the *Data Analysis Guide* for more information.

- “To prepare the Agilent MassHunter Triple Quad LC/MS computer” on page 43

Confirm that all MS methods used in Sequences-mode operation are present in the folder **D:\MassHunter\methods\RapidFire**, as required for Agilent MS integration.

- “To prepare the ABI/Sciex Triple Quad MS computer” on page 45
See “Further setup for ABI/Sciex Triple Quad MS computer only” below.

Further setup for ABI/Sciex Triple Quad MS computer only

For Sequences-mode operation:

- 1 Copy the RapidFire Template Project in **D:\Analyst\Projects**. This folder is provided by Agilent and contains the following files:
 - **Template1.dab** (data acquisition batch)
 - **Template1.dam** (data acquisition method)
 - **Equilibrate.dam**
- 2 Paste the project in the same folder (**D:\Analyst\Projects**), then rename it.
- 3 In Analyst:
 - a Select the newly created project as the current one.
 - b Open, convert and save the data acquisition batch **Template1.dab** in the new project.

To set parameters in the RapidFire method

- 1 Set the parameters for your analysis on the RapidFire 365 High-throughput Mass Spectrometry System.

The parameters outlined in green in the following figure make up a RapidFire method saved in a **.RFCFG** file.

Sipper Settings

☒ ☒ Wash Needle Between Sips

Sip Height (mm) 2 2

Blank Injections Between Wells 0 0

Flush Now Vacuum Level (kPa) NA

RapidFire Cycle Durations

	State	Edit Time (ms)	Time (ms)
	Aspirate	600	600
	Load/Wash	3000	3000
	Extra Wash	1000	1000
	Elute	3000	3000
	Re-equilibrate	500	500

Pump Settings

Pump 1

1.5 0 mL/min

10 10 B%

0 0 C%

0 0 D%

0.02 MPa

Pump 2

1.25 0 mL/min

0 0 B%

0 0 C%

0 0 D%

0.03 MPa

Pump 3

1.25 0 mL/min

80 80 B%

0 0 C%

0 0 D%

0.01 MPa

Pump 4

0 0 mL/min

0 0 B%

0 0 C%

0 0 D%

Miscellaneous Settings

☒ ☒ Use Plate Handler

☒ ☒ Use Barcode Scanner

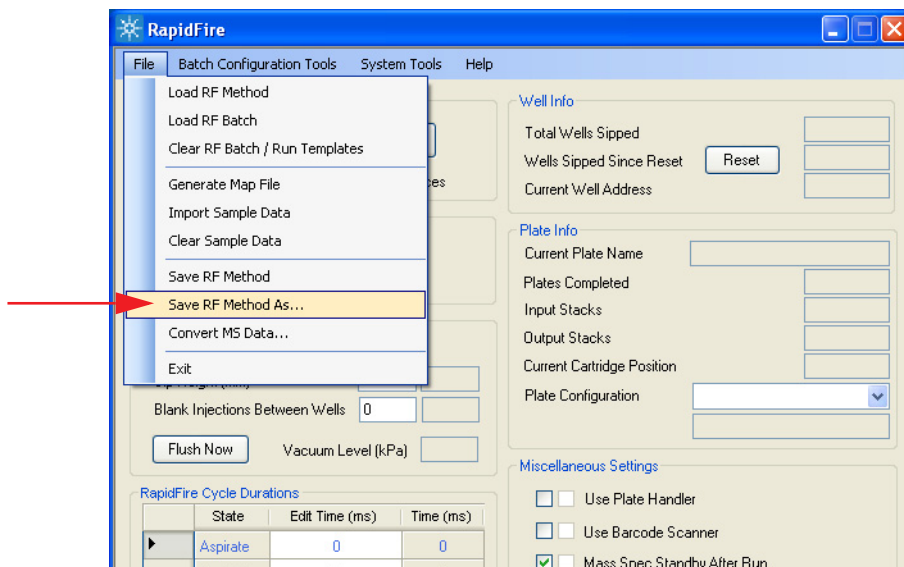
☐ Mass Spec Standby After Run

Plates Between Flushes 1 1

Missed Sip Tolerance 10 10

- 2 In the **Sipper Settings** area of the screen:
 - a Mark the **Wash Needle Between Sips** option to wash the sipper tube once after aspiration of the sample from each well. The tube is washed first in the aqueous, then in the organic wash chimney, for 300 ms in each.
 - b In the **Sip Height** field, type the number of millimeters from the *lowest position* of the sipper tube to the bottom of the well. Enter a value of 1.0 mm or greater. The sipper tube (in V1P3) travels down from Safe Z into the sample well until *either*:

- The sip sensor (in V1P4) optically detects the presence of fluid and triggers the actuation of V1, which causes the transition between State # 1 (Aspiration) and State # 2 (Load/Wash), *or*
 - The z-position of the sipper tube reaches the set **Sip Height**.
- c** Enter a value > 0 for **Blank Injections Between Wells** to have the system do more cycles in the organic wash station *between wells*. This setting helps to reduce or prevent carryover between samples.
- 3** In the **RapidFire Cycle Durations** area, set the valve timing, as per assay protocol for States 1 - 5 (**Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, and **Re-equilibrate**).
- Set the value of **Aspirate** to at least 600 ms for reliable sip sensor triggering and sample sipping.
- 4** In the **Pump Settings** area at the bottom of the screen, set pump flow rates and solvent compositions for your assay.
- 5** Click **Apply**.
- 6** To save the parameter settings made in the previous steps for future use, click **File > Save RF Method As**. The settings are stored in a **.rfcfg** file.

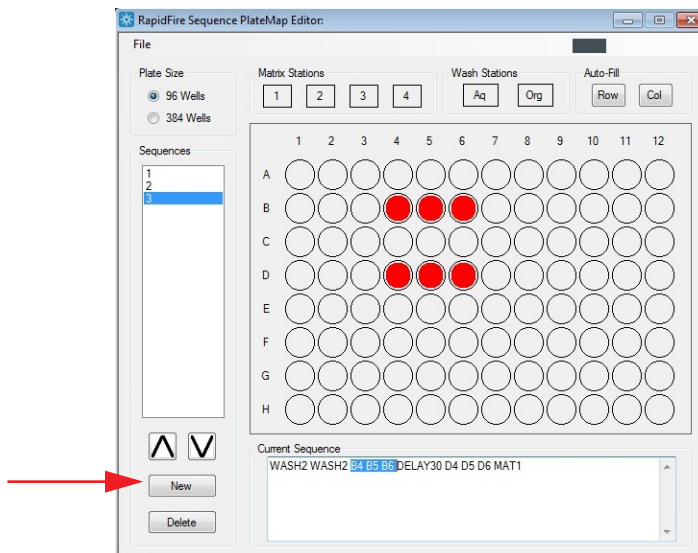


4 Operation in Sequences Mode

To edit the plate map

To edit the plate map

- 1 Click **Batch Configuration Tools > Plate Map Editor**.
- 2 For **Plate Size**, select 96- or 384-wells.
- 3 To create a sequence for a plate map, click **New**.



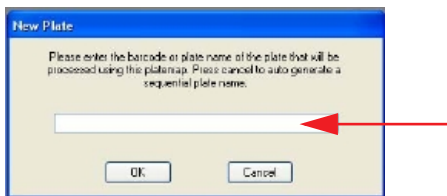
- 4 To create your sequence, click the following areas or buttons:
 - Well positions in the plate diagram
 - Matrix station number (1-4)
 - Wash station (Aqueous or Organic)
- 5 To create another sequence for this plate map, repeat Steps 3 and 4.
 - A single plate map can contain several sequences, which are listed in the **Sequences** box on the left side of the window.
 - The **Current Sequence** is shown at the bottom of the window.
- 6 To save the settings made in the previous steps for future use, click **File > Save Platemap As**. The settings are stored in a **.rfmap** file.
- 7 (*Optional*) You can enter a special well designation for each sequence to insert a pause in RapidFire System data acquisition. Enter this well number

in the **Current Sequence** text box in the format **DELAY#**, where **#** is an integer that specifies the number of seconds of delay.

Example For a sequence such as “**A1 A2 A3 A4 delay30**” ..., after wells A1 through A4 are injected, the system pauses for 30 seconds, then proceeds with the next sequence listed in the Batch Editor.

To edit the batch

- 1 Click **Batch Configuration Tools > Batch Editor**.
- 2 To create a batch, click **Add**.
- 3 Select the plate map (**.RFMAP** file) to be added to this batch.
Plate maps are created as described in “[To edit the plate map](#)” on page 66.
 - a When the New Plate window appears, enter the plate name or scan the barcode of the plate.



- b Click **OK**.

Tip If you click **Cancel** without entering a plate name, then the plate is named Plate1, Plate2, Plate3, and so on, by default.

- 4 Select the **MS Integration** software that the RapidFire System run is to be synchronized with, such as **MassHunter QQQ**.
 - If you select Analyst, also enter or select a Project to use.
 - *Integration with Thermo Fisher MS is not currently supported.*

4 Operation in Sequences Mode

To edit the batch

- 5 For each sequence (each line) of the batch:
 - a Double-click the cell in the **RF Method** column, select the desired method (**.RFCFG** file), and click **Open**.
 - b Select the **Cartridge** for each sequence from the list. The cartridge **Type** must match the cartridge types in the Cartridge Changer dialog box.
 - c Select the **MS Method** for each sequence from the list. Alternatively, you can type in the name and extension of the desired method (**.M file** for Agilent MS integration or **.DAM** file for AB/Sciex MS integration).

**To save time,
Copy/Paste
values in table**

- To copy a value from a selected cell, use **Ctrl + C**.
- Highlight other cells that you want to have the same value.
- To paste in the value, use **Ctrl + V**.

- 6 Save this batch, so that it will be available for future use. To save the batch, click **File > Save RF Batch As**. The settings are stored in a **.RFBAT** file.

Tip To modify an existing batch:

- a Click **File > Load RF Batch**.
- b Select the batch of interest (**.RFBAT** file).
- c Double-click the **Plate Name** you want to modify.
- d Save the batch.

To set run parameters

Verify that parameters settings are as follows in the RapidFire window.

The screenshot shows the RapidFire window with the following settings:

- Plate Info:**
 - Current Plate Name: (empty)
 - Plates Completed: 0
 - Input Stack: NA
 - Output Stack: NA
 - Current Column Position: Unknown
 - Plate Configuration: StandardPlate_96 (selected in dropdown)
- Miscellaneous Settings (highlighted with a green border):**
 - ☐ Use Plate Handler
 - ☐ Use Barcode Scanner
 - ☒ Mass Spec Standby After Run
 - Plates Between Flushes: 4 / 4
 - Missed Sip Tolerance: 10 / 10

- 1 In the **Plate Info** area, confirm that the correct **Plate Configuration** is selected (96-well or 384-well).
- 2 In the **Miscellaneous Settings** area of the screen:
 - a Mark whether to:
 - **Use Plate Handler**
 - **Use Barcode Scanner**
 - For Triple Quad, put the **Mass Spec on Standby After the Run**.
 - For QTOF, *clear* the option to put the **Mass Spec on Standby After the Run**, as this mode is *not* recommended for TOF applications.
 - b Set the **Plates Between Flushes** as follows:
 - For 96-well plates, use **4**.
The sipper tube flushes for 1 minute after *every 4 plates*.
 - For 384-well plates, use **1**.
The sipper tube flushes for 1 minute after *every plate*.
 - c To stop RapidFire System operation after the sip sensor detects the specified number of consecutive empty wells, set the **Missed Sips Tolerance** to a value > 0.
- 3 Click **Apply** to set the desired values.

To run a batch in Sequences mode

- 1 Click **File > Load RF Batch**.
- 2 Click the **Play** button in the upper left area of the screen
 - a From the list of plates, select the first plate to read.
 - b Click the **Play** button again.
 - c Verify that the sipper tube aspirated the first samples.
 - d Verify that the mass spectrometer generated a signal.



NOTE

Close the doors while operating the RapidFire System to prevent possible injury. The system does not operate with the doors open.

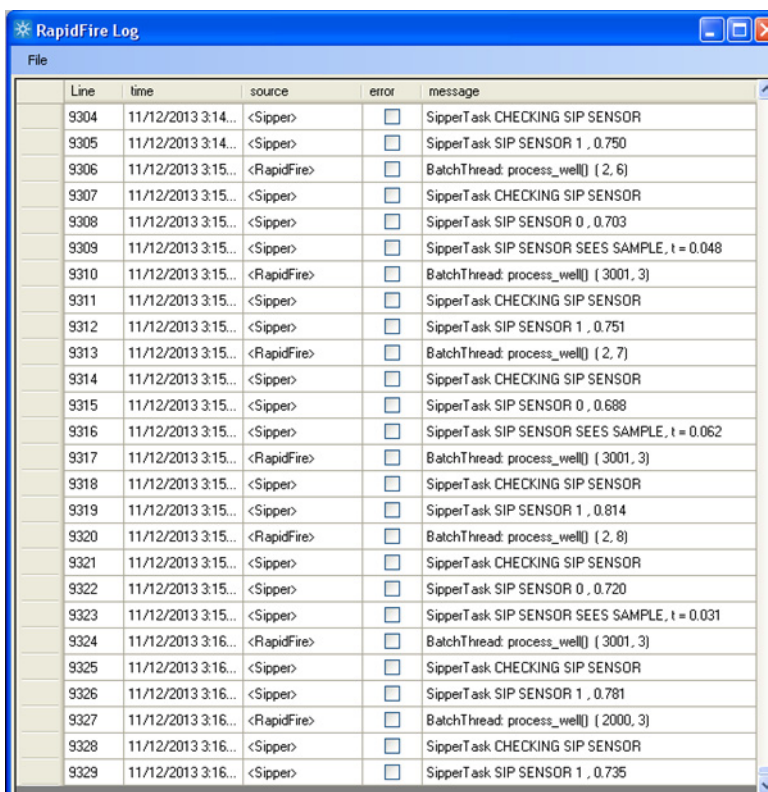
- 3 If you are loading plates manually, then when a plate is completed, you are prompted to load a new plate.
 - a If you have more plates to read, then:
 - Remove the previous plate.
 - Position your next plate on the deck as described in [“To load plates manually”](#) on page 58.
 - When prompted, select the next plate from the list.If you do not enter a plate name in the next 30 minutes, then a time-out feature automatically puts the RapidFire System in stand-by mode to keep solvent from being wasted.
 - b Operation stops when all plates in this set have been run.
- 4 To monitor a run, use the following features:
 - a In the **Well Info** area of the screen, view the following information:
 - the number of wells sipped in the lifetime of your RapidFire-MS System
 - the number of wells sipped since the last reset
 - the location of the Current Well Address (such as [1,7] for position A7).
 - b To monitor the progress of your RapidFire run, click **System Tools > View Injection List** as shown in the following example:

Injection List														
Barcode	Plate Position	Injection	Sequence	Siptime	Sir	Acquisition Time	Operator Name	Instrument Name	Cartridge	Cartridge Injection	Method	RF Method	Dat File	
Control_1312	WASH2	1	1	3.338	1	2012-05-31T12:35:53Z	Dorian	RapidFire 360...	A	313	Immuno Panel.m	Agilent(basic)		
Control_1312	B1	2	1	13.166	0	2012-05-31T12:36:09Z	Dorian	RapidFire 360...	A	314	Immuno Panel.m	Agilent(basic)		
Control_1312	B2	3	1	22.744	0	2012-05-31T12:36:18Z	Dorian	RapidFire 360...	A	315	Immuno Panel.m	Agilent(basic)		
Control_1312	B3	4	1	32.369	0	2012-05-31T12:36:27Z	Dorian	RapidFire 360...	A	316	Immuno Panel.m	Agilent(basic)		
Control_1312	B4	5	1	41.993	0	2012-05-31T12:36:37Z	Dorian	RapidFire 360...	A	317	Immuno Panel.m	Agilent(basic)		
Control_1312	B5	6	1	51.352	0	2012-05-31T12:36:47Z	Dorian	RapidFire 360...	A	318	Immuno Panel.m	Agilent(basic)		
Control_1312	B6	7	1	60.962	0	2012-05-31T12:36:56Z	Dorian	RapidFire 360...	A	319	Immuno Panel.m	Agilent(basic)		
Control_1312	B7	8	1	70.274	0	2012-05-31T12:37:06Z	Dorian	RapidFire 360...	A	320	Immuno Panel.m	Agilent(basic)		
Control_1312	B8	9	1	79.820	0	2012-05-31T12:37:16Z	Dorian	RapidFire 360...	A	321	Immuno Panel.m	Agilent(basic)		
Control_1312	B9	10	1	89.211	0	2012-05-31T12:37:25Z	Dorian	RapidFire 360...	A	322	Immuno Panel.m	Agilent(basic)		
Control_1312	B10	11	1	98.304	0	2012-05-31T12:37:34Z	Dorian	RapidFire 360...	A	323	Immuno Panel.m	Agilent(basic)		
Control_1312	WASH2	12	1	106.632	1	2012-05-31T12:37:42Z	Dorian	RapidFire 360...	A	324	Immuno Panel.m	Agilent(basic)		
Control_1312	E2	13	2	3.016	0	2012-05-31T12:38:11Z	Dorian	RapidFire 360...	A	325	Immuno Control S...	Agilent(long-slow-elution)		
Control_1312	E3	14	2	14.984	0	2012-05-31T12:38:24Z	Dorian	RapidFire 360...	A	326	Immuno Control S...	Agilent(long-slow-elution)		
Control_1312	E4	15	2	26.671	0	2012-05-31T12:38:35Z	Dorian	RapidFire 360...	A	327	Immuno Control S...	Agilent(long-slow-elution)		
Control_1312	WASH2	16	2	37.342	1	2012-05-31T12:38:46Z	Dorian	RapidFire 360...	A	328	Immuno Control S...	Agilent(long-slow-elution)		
Immuno_1509	WASH2						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A1						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A2						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A3						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A4						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A5						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A6						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A7						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		
Immuno_1509	A8						Dorian	RapidFire 360...	A		Immuno Panel.m	Agilent(basic)		

- c Click **System Tools > View Log** to monitor the progress of your RapidFire run as shown in the following example:

4 Operation in Sequences Mode

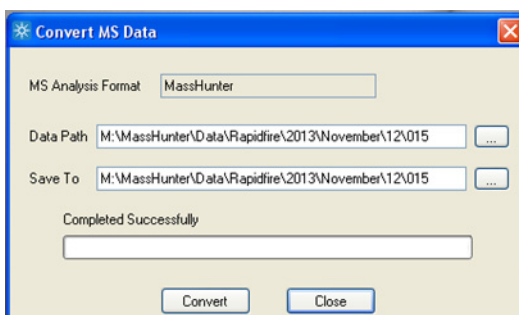
To run a batch in Sequences mode



The screenshot shows the 'RapidFire Log' window with a table of log entries. The table has five columns: Line, time, source, error, and message. The entries show a sequence of operations including SipperTask CHECKING SIP SENSOR, SipperTask SIP SENSOR 1, 0.750, BatchThread: process_well() (2, 6), SipperTask CHECKING SIP SENSOR, SipperTask SIP SENSOR 0, 0.703, SipperTask SIP SENSOR SEES SAMPLE, t = 0.048, BatchThread: process_well() (3001, 3), SipperTask CHECKING SIP SENSOR, SipperTask SIP SENSOR 1, 0.751, BatchThread: process_well() (2, 7), SipperTask CHECKING SIP SENSOR, SipperTask SIP SENSOR 0, 0.688, SipperTask SIP SENSOR SEES SAMPLE, t = 0.062, BatchThread: process_well() (3001, 3), SipperTask CHECKING SIP SENSOR, SipperTask SIP SENSOR 1, 0.814, BatchThread: process_well() (2, 8), SipperTask CHECKING SIP SENSOR, SipperTask SIP SENSOR 0, 0.720, SipperTask SIP SENSOR SEES SAMPLE, t = 0.031, BatchThread: process_well() (3001, 3), SipperTask CHECKING SIP SENSOR, SipperTask SIP SENSOR 1, 0.781, BatchThread: process_well() (2000, 3), SipperTask CHECKING SIP SENSOR, and SipperTask SIP SENSOR 1, 0.735.

Line	time	source	error	message
9304	11/12/2013 3:14...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9305	11/12/2013 3:14...	<Sipper>	☐	SipperTask SIP SENSOR 1 , 0.750
9306	11/12/2013 3:15...	<RapidFire>	☐	BatchThread: process_well() (2, 6)
9307	11/12/2013 3:15...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9308	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR 0 , 0.703
9309	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR SEES SAMPLE, t = 0.048
9310	11/12/2013 3:15...	<RapidFire>	☐	BatchThread: process_well() (3001, 3)
9311	11/12/2013 3:15...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9312	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR 1 , 0.751
9313	11/12/2013 3:15...	<RapidFire>	☐	BatchThread: process_well() (2, 7)
9314	11/12/2013 3:15...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9315	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR 0 , 0.688
9316	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR SEES SAMPLE, t = 0.062
9317	11/12/2013 3:15...	<RapidFire>	☐	BatchThread: process_well() (3001, 3)
9318	11/12/2013 3:15...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9319	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR 1 , 0.814
9320	11/12/2013 3:15...	<RapidFire>	☐	BatchThread: process_well() (2, 8)
9321	11/12/2013 3:15...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9322	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR 0 , 0.720
9323	11/12/2013 3:15...	<Sipper>	☐	SipperTask SIP SENSOR SEES SAMPLE, t = 0.031
9324	11/12/2013 3:16...	<RapidFire>	☐	BatchThread: process_well() (3001, 3)
9325	11/12/2013 3:16...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9326	11/12/2013 3:16...	<Sipper>	☐	SipperTask SIP SENSOR 1 , 0.781
9327	11/12/2013 3:16...	<RapidFire>	☐	BatchThread: process_well() (2000, 3)
9328	11/12/2013 3:16...	<Sipper>	☐	SipperTask CHECKING SIP SENSOR
9329	11/12/2013 3:16...	<Sipper>	☐	SipperTask SIP SENSOR 1 , 0.735

- d For data analysis by the MassHunter Workstation Quantitative Analysis software, create individual peak data files as follows:
- Click **File > Convert MS Data**.



The screenshot shows the 'Convert MS Data' dialog box. It has a title bar with a star icon and a close button. The 'MS Analysis Format' is set to 'MassHunter'. The 'Data Path' and 'Save To' fields both contain the path 'M:\MassHunter\Data\Rapidfire\2013\November\12\015'. Below these fields is a 'Completed Successfully' label and a text box. At the bottom are 'Convert' and 'Close' buttons.

MS Analysis Format: MassHunter

Data Path: M:\MassHunter\Data\Rapidfire\2013\November\12\015

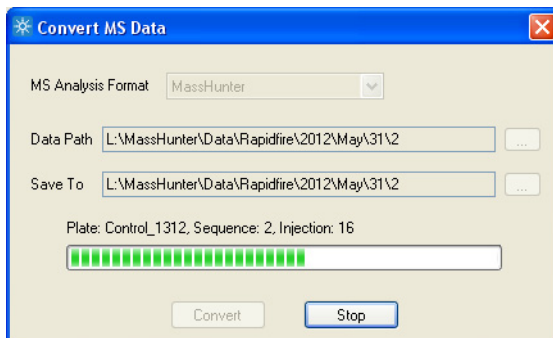
Save To: M:\MassHunter\Data\Rapidfire\2013\November\12\015

Completed Successfully

Convert Close

- Select the whole-experiment data directory (**Data Path**) of the files to convert. The data path of the most recent run is automatically displayed.
- Enter a **Save To** location for the converted single-injection data files.
- Click **Convert**.

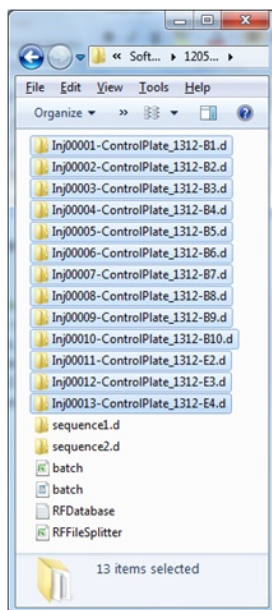
The following progress dialog box is displayed:



- When the conversion is done, you can see that individual data files have been created and saved in the specified location as shown in the following example:

4 Operation in Sequences Mode

To run a batch in Sequences mode



- To analyze the data, refer to MassHunter Workstation Quantitative Analysis software manuals and online Help.

To pause a run

Use this procedure to suspend a run temporarily, such as when you change buffer solutions or do other routine maintenance.

1 Click the **Pause** button .

- The cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, **Re-equilibrate**, and **Needle Washes** is completed for the last sample injected, then the RapidFire System pauses operation.
- The mass spectrometer continues to collect “baseline” data.
- The RapidFire System pumps continue to run.

2 To resume operation after pausing, click the **Play** button .

- The system starts up where it left off and data is saved to the current data file.
- The remaining samples are run in the original order.

To stop a run

1 Click the **Stop** button .

- The cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, **Re-equilibrate**, and **Needle Washes** is completed for the last sample injected, then the RapidFire System stops operation.
- Data acquisition is stopped and the current data file is closed.

4 Operation in Sequences Mode

To make an emergency stop

To make an emergency stop

CAUTION

Use the E-STOP procedure only during an emergency.

Otherwise, use either of these procedures to stop the RapidFire System:

[“To pause a run”](#) on page 75, or

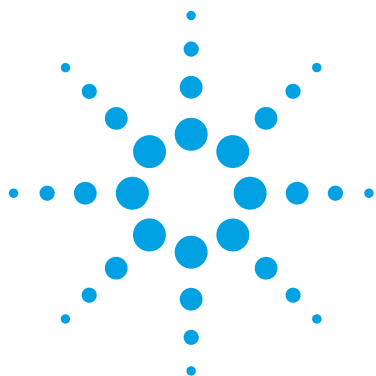
[“To stop a run”](#) on page 75.

- 1 Press the physical emergency stop button (labeled **E-STOP**), which cuts power of the entire system.



Opening an interlock door of the RapidFire System also stops motion.

- 2 To restart the system, pull up the **E-STOP** button to re-establish power.



5 Technical Specifications and Parts

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This chapter provides information about technical specifications, parts, and consumables for the RapidFire 365 High-throughput Mass Spectrometry System.



Technical Specifications & Installation Requirements

Authorized Agilent Technologies field representatives will install your RapidFire 365 High-throughput Mass Spectrometry System in the location that you specify.

Pre-installation requirements

Before the installation, check that you have the following available:

- 2.5 x 2.5 meters (8 x 8 feet) of space in which to place the RapidFire 365 High-throughput Mass Spectrometry System, mass spectrometer, and computer controller
- A house vacuum or a stand-alone vacuum pump
- Two power sockets are required for operating a RapidFire 365 system. *One* of the following types of circuits will be used, depending on your location:
 - a dedicated 120 V, 15 A circuit (North America)
 - a dedicated 230 V, 10 A circuit (Europe)
 - a dedicated 100 V, 15 A circuit (Japan)
- A well-calibrated mass spectrometer

WARNING

The RapidFire machine weighs approximately 475 kg (1,045 lbs).

Do *not* attempt to lift the RapidFire platform. Instead, move the RapidFire machine by wheeling its platform.

During operation, be sure to lock down the casters of the RapidFire platform.

Electrical ratings

RapidFire 365 High-throughput Mass Spectrometry System is rated for operation:

- 100 -240 V, 50/60Hz, max. 615 W (power strip) *and*
- 120 V, 50/60 Hz, 12 A (UPS SMT1000) *or*
- 220-240 V, 50/60 Hz, max 7.25 A (UPS SMT1000I)

WARNING

Route the main supply cable in such a way as to minimize the risk of a tripping hazard. Agilent recommends the use of mechanical protection, for instance a rubberized cable guard on the floor to cover and protect the main supply and other cables.

Environmental operating ranges

Use the RapidFire System at the following conditions:

- **Pollution degree:** 2
- **Installation category:** II
- **Temperature:** 4°C to 35°C
- **Relative humidity:** 20% to 90%, non-condensing
- **Environment:**
 - non-explosive surroundings
 - indoor use only
 - laboratories use only
- **Altitude:** 2000 meters

Recorded noise level for the RapidFire System unit is below 70 dbA.

Warranty

The warranty for the RapidFire System is for one year. Parts, labor, and travel are included.

The mass spectrometer is covered under a separate by warranty from its manufacturer.

System modifications

Do not modify the system.

Alterations of the instrument without approval from Agilent Technologies will void the manufacturer's warranty. Agilent Technologies is not responsible for mishaps that are caused by modifications to the RapidFire System, which are not made by Agilent employees or its authorized agents.

Safety information

Safety information is provided in the *Safety Guide*.

Materials

Agilent-supplied items

RapidFire System contents:

- RapidFire 365 High-throughput Mass Spectrometry System instrument
- Motion control computer
- Three digitally controlled high-pressure fluidic pumps
- Digital peristaltic pump
- Uninterruptible power supply (UPS) for the RapidFire System
- Sipper flush valve
- Two barcode scanners
- Waste collection vessels
- BenchBot microplate handler
- 12-slot cartridge changer
- Sip sensor

Consumables:

- Standard RapidFire solid-phase extraction (SPE) cartridges.

Purchase custom assay-specific cartridges from Agilent.

See Also [“RapidFire System Cartridges”](#) on page 83.

Customer-supplied items

- Assay-specific solvents, LC/MS grade, filtered by the manufacturer.

WARNING

Handle solvents safely as described in the *Safety Guide*. Contact the manufacturer for material safety data sheets (MSDS) for all materials that you use, including:

- water
- methanol
- acetonitrile
- acetone
- formic acid
- TFA: trifluoroacetic acid
- TEA: triethylamine
- ammonium acetate
- isopropyl alcohol
- chloroform

-
- 1 liter graduated cylinder
 - Pipettes and pipette tips
 - Sample 96- and 384-well plates with standard bar codes

RapidFire System Cartridges

Part number	Type	Packing material
G9203A	A	C4
G9204A	B	Cyano
G9205A	C	C18
G9206A	D	Graphitic carbon
G9207A	E	C8
G9208A	F	Phenyl
G9209A	H1	HILIC
G9210A		Custom
G9211A	0	Blank
G9525A	A2	C4

RapidFire System Flow Path

“State #1: Aspirate” on page 84

“State #2: Wash/Load” on page 85

“State #3: Extra Wash” on page 86

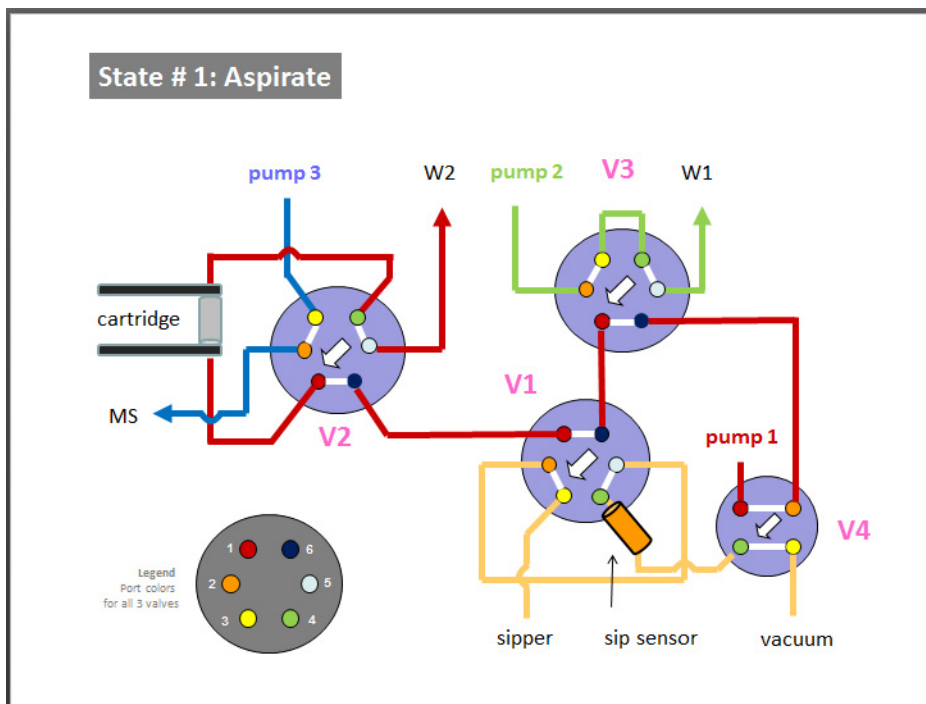
“State #4: Elute” on page 87

“State #5: Re-equilibrate” on page 88

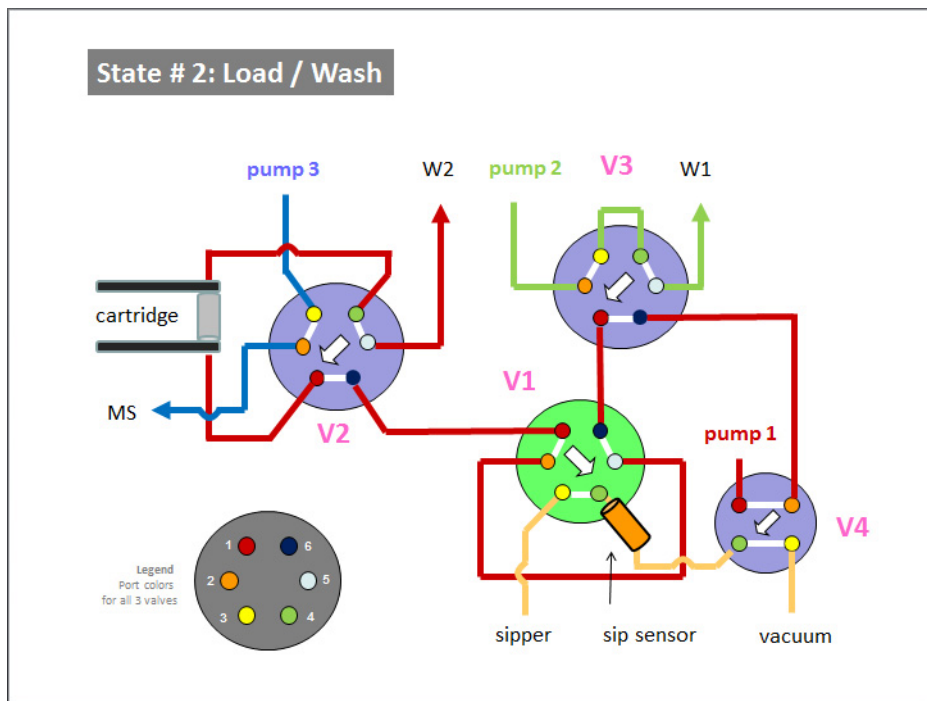
“Physical colors of the tubing” on page 90

“Valve positions” on page 90

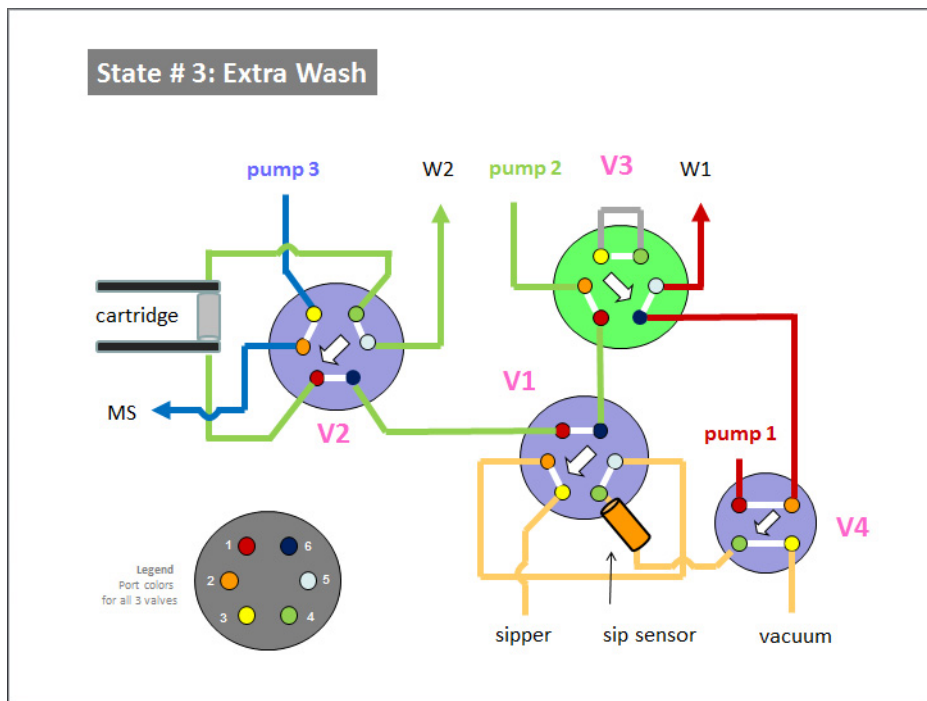
State #1: Aspirate



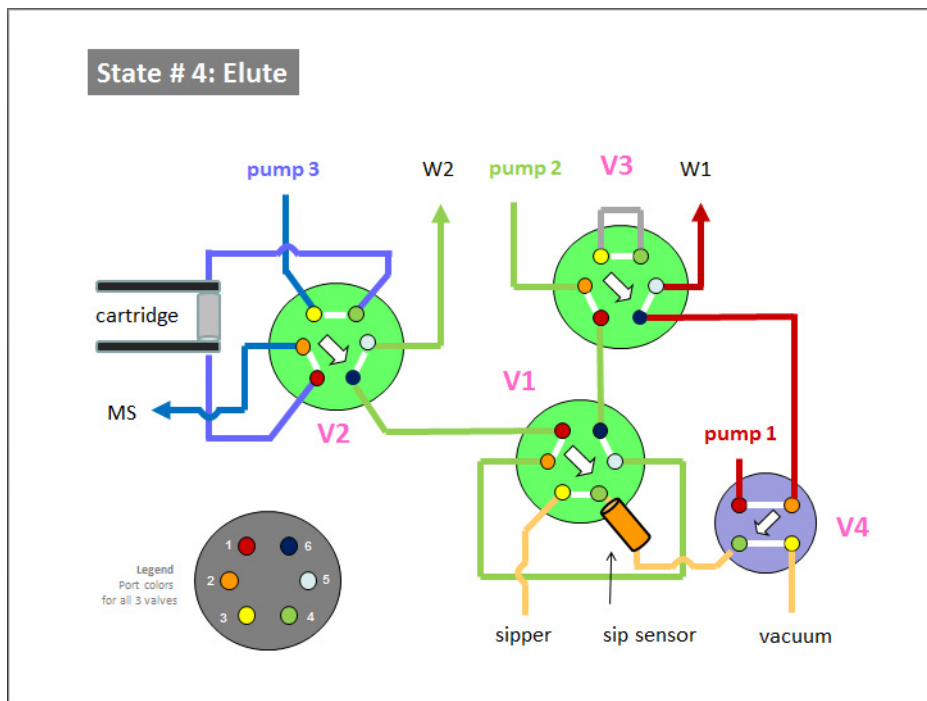
State #2: Wash/Load



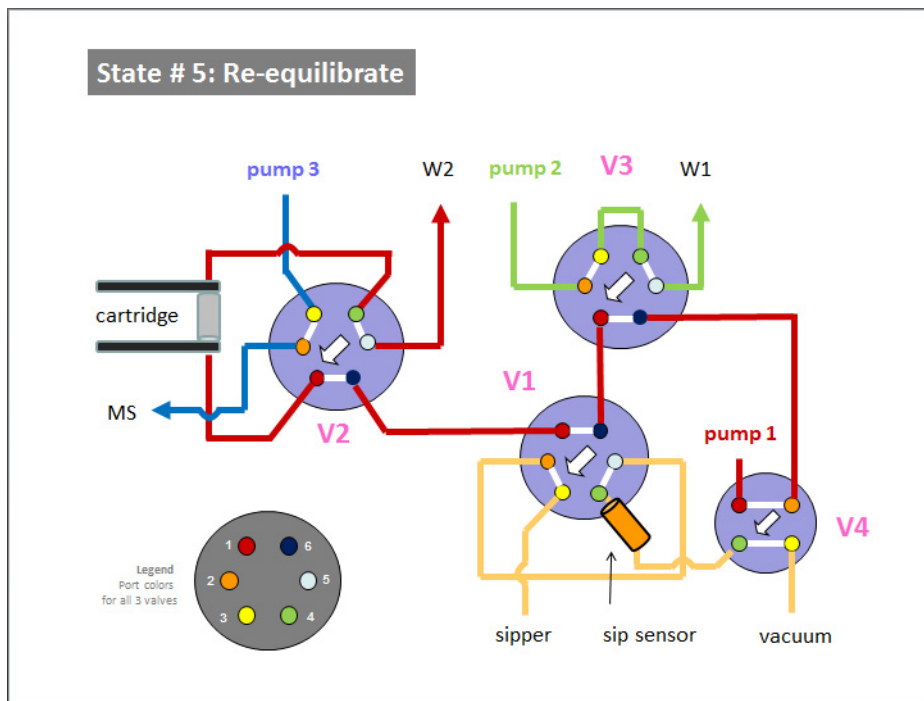
State #3: Extra Wash



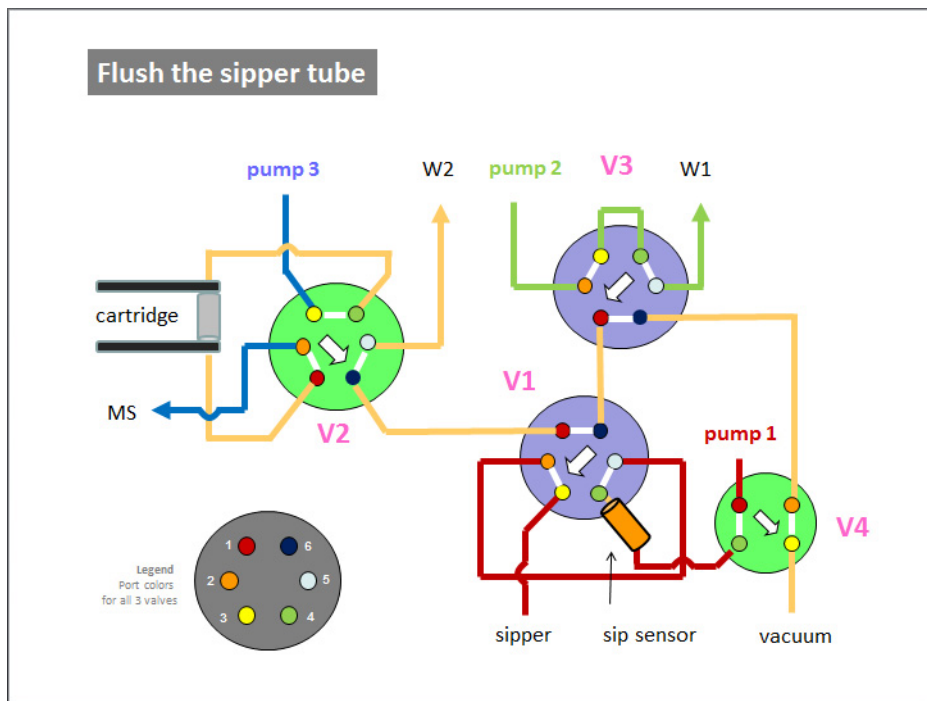
State #4: Elute



State #5: Re-equilibrate



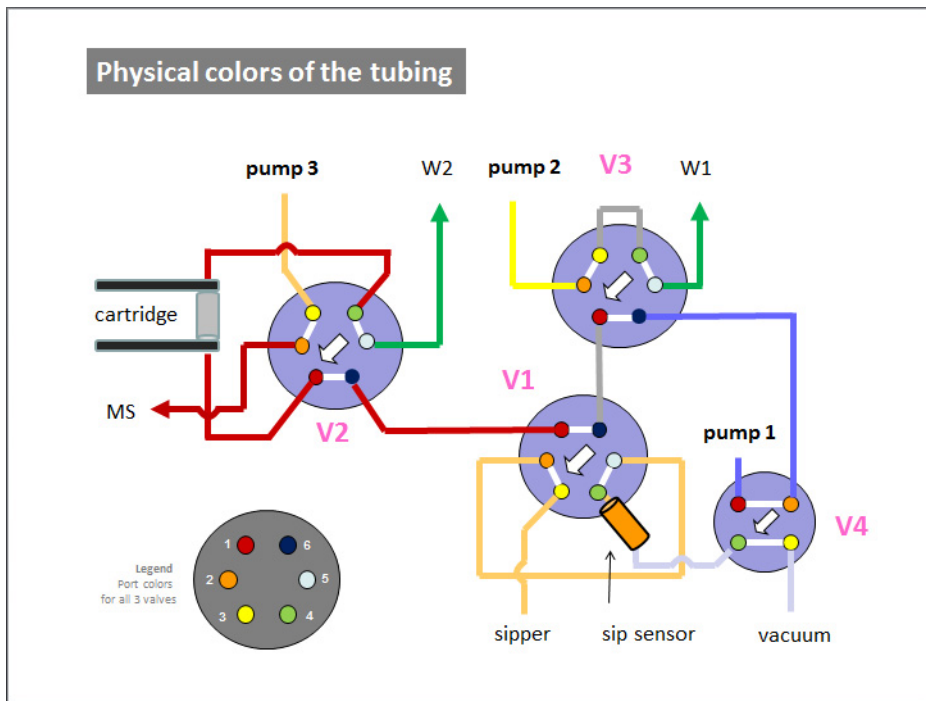
Flush the sipper tube



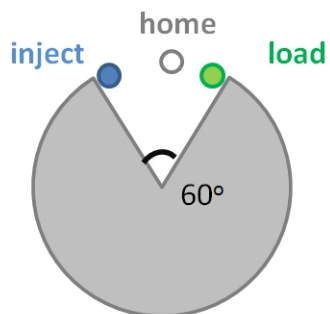
5 Technical Specifications and Parts

Physical colors of the tubing

Physical colors of the tubing



Valve positions



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In This Book

This guide gives information about how to use the RapidFire 365 High-throughput Mass Spectrometry System.

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