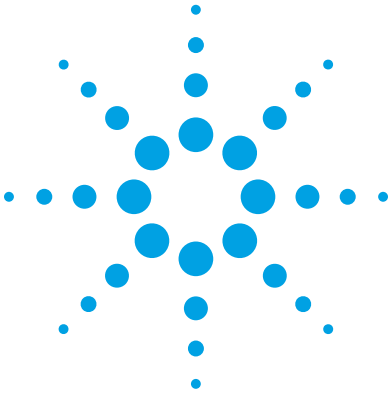

Agilent G9531A RapidFire 365 High-throughput Mass Spectrometry System

User Guide

For Research Use Only. Not for use in diagnostic procedures.

Agilent Technologies

Notices

Manual Part Number

G9531-90003

Edition

Revision A, August 2017

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051 USA

Software Revision

This guide is valid for the RapidFire Control Software version 4.3.

Instrument Manufacturing

Manufactured by Agilent Technologies Singapore Pte. Ltd. No. 1 Yishun Avenue 7, Singapore 768923

Operating Temperature: 4°C to 35°C

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CAUTION

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WARNING

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Content

Contents

1 System Preparation

This chapter contains information to help you start up and turn off the 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.

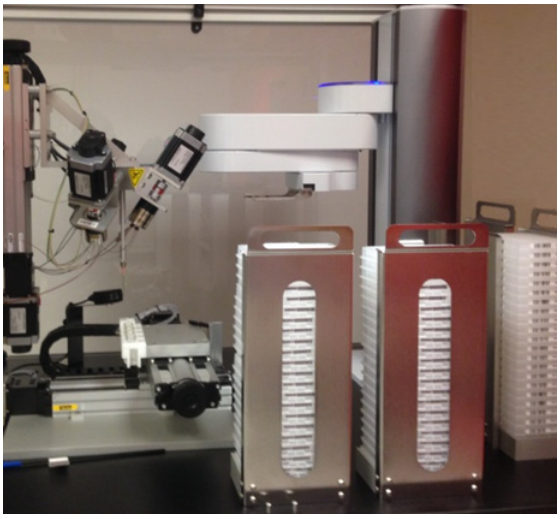


Figure 1 BenchBot sample handler and input stacks.

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.

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.

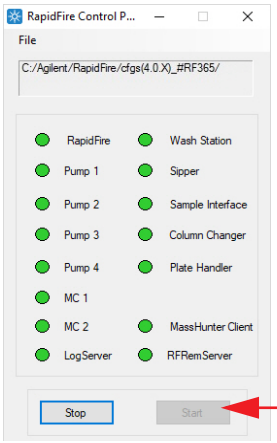
To start the software

1 Double-click the **RapidFire Control Panel** desktop icon.

2 Click **File > Choose CFGs**.

3 Select the configuration folder under **C:\Agilent\RapidFire**.

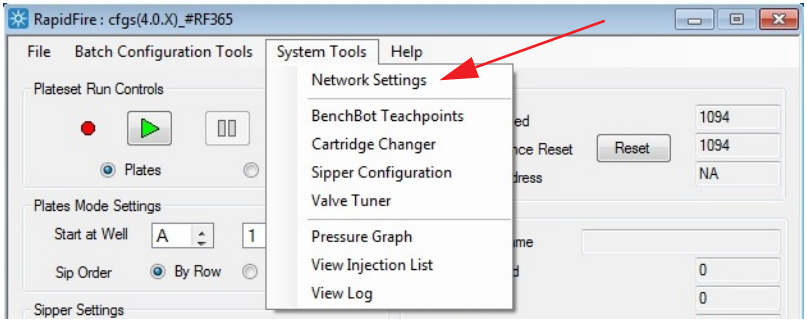
4 Click **Start** in the Control Panel dialog box.



The icons turn green as the system components start up.

5 Double-click the **RapidFire UI** desktop icon.

6 Click **System Tools > Network Settings**.

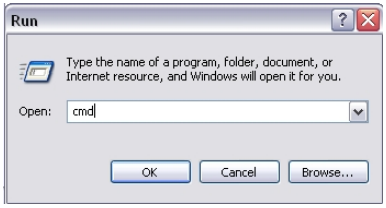


7 Verify that the IP addresses for the RapidFire System computer and the mass spectrometer computer are correct.

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**.

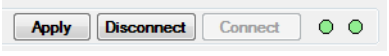


c When a command window opens, type **ipconfig -all** and press **Enter**.

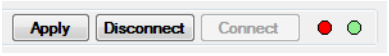
The IP address is displayed in the window.

8 To connect to the program server, click **Connect** in the main window.

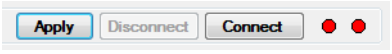
The dots next to the **Connect** button turn green.



If one dot is red, the system is connected, but the door is open or the E-stop is pressed.



If both dots are red, the system is not connected.



To home the cartridge changer and replace the cartridge

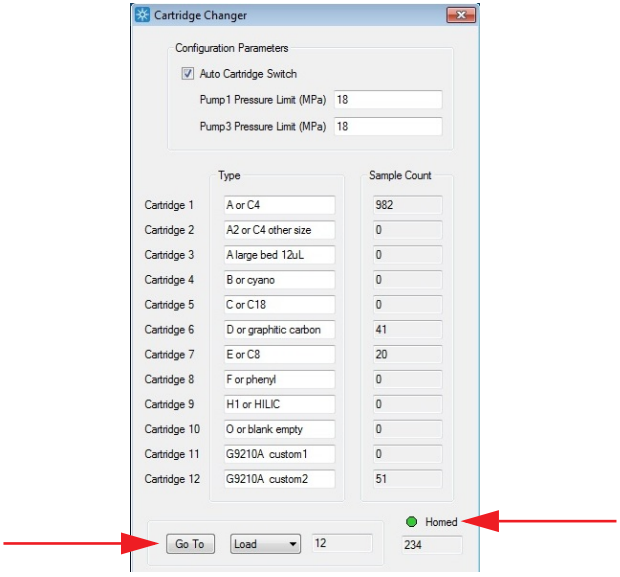
1 To open the Cartridge Changer dialog box, click **System Tools > Cartridge Changer**.

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.

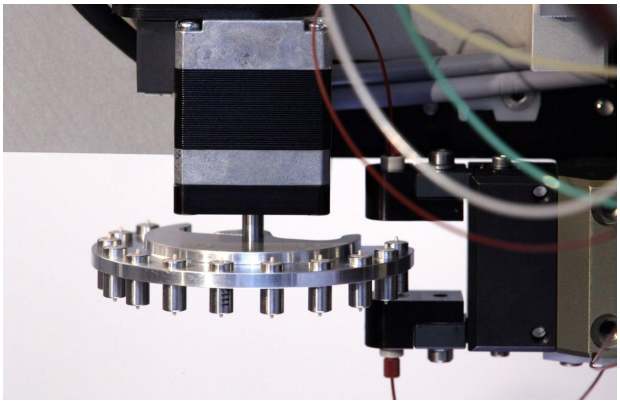


   Be sure that a cartridge is present in the Cartridge 1 slot, as the cartridge changer travels to this position after the homing routine is complete.

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.



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 75 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, make sure you have an empty example plate in either the 96- or 384-well format.

1 Click **System Tools > Sipper Configuration** to open the Sipper Configuration wizard.

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:

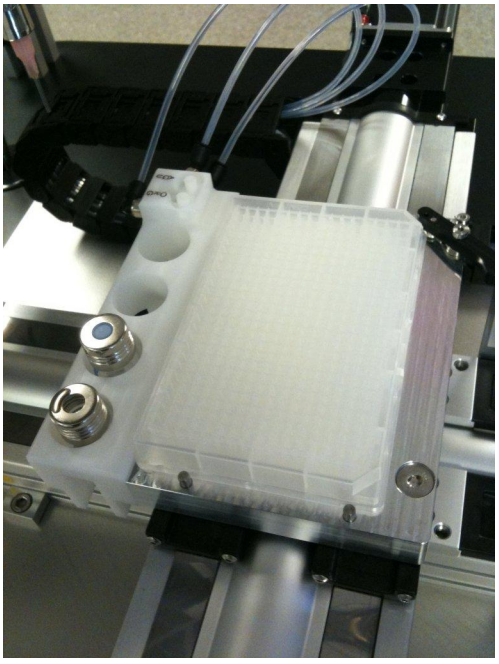
   a Close the wizard.

   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 Z-axis so that the bottom of the sipper needle clears all obstacles (such as the plate, wash station, and matrix bottle) by about 0.5 cm (1/4 inch), regardless of where the stage moves along the X- or Y-axis.

b Click **Set**, and then click **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.

  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**, and then click **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**, and then click **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.

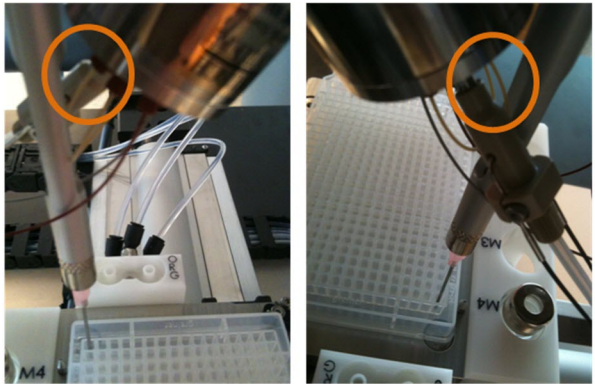
c Click **Set**, and then click **Next**.

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.



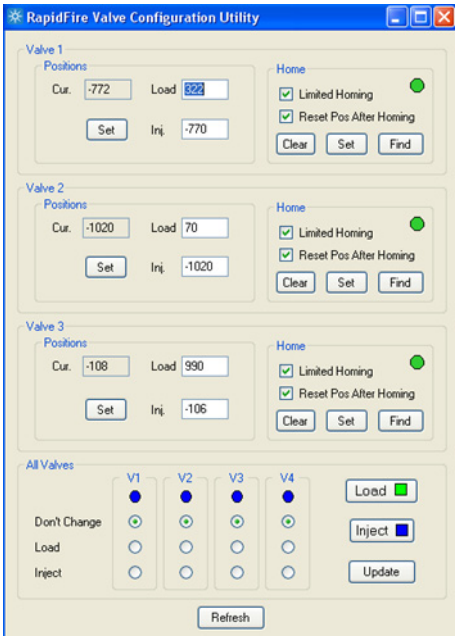
10 Repeat step 9 to define positions A-1 and A-24 of the 384-well plate or H1, A1, and A24 of a 96-well plate.

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**, and then close the Sipper Configuration Wizard.

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 light for any valve is yellow, or if the Home indicator is red, then click **Find** for that valve to reset the valve position.

If properly positioned, all valve Home indicators are green, and **Cur.** under **Positions** shows **0**.

If a valve does not reset to its home position:

a Press the **E-STOP** button.

b Rotate the valve as far counterclockwise as it will go.

c Disengage (reset) the **E-STOP** button.

d Press **Home** again.

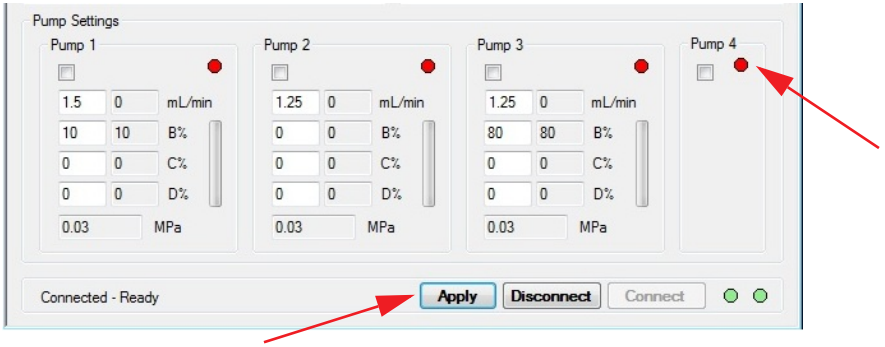
4 Check that switching the valves from their load to their inject position is smooth and quiet.

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.

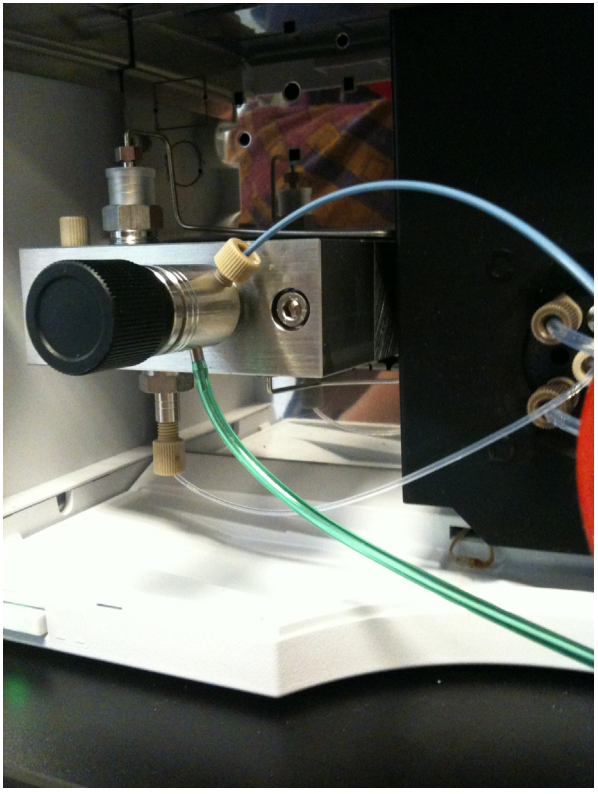
- You can adjust the flow rate of the peristaltic pump manually by pressing the up and down arrows on its control panel.
- If the peristaltic has been off for hours, then the organic channel takes a few minutes longer than the aqueous one to reach its chimney steadily.

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.*

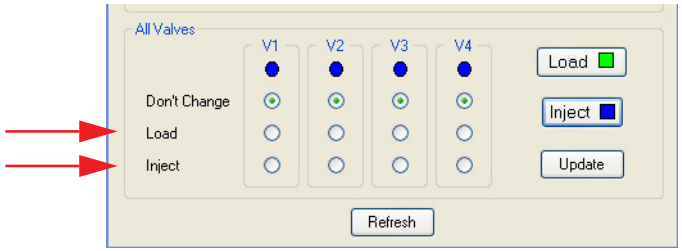
7 Check the pressure readings, keeping in mind the following:

   - 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.



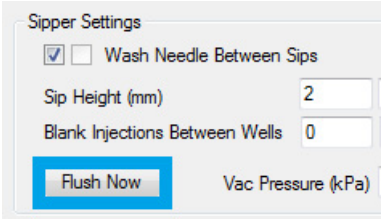
Here are some typical values:

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 and 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.

To prepare the MassHunter Workstation for TOF or Q-TOF LC/MS

Before you begin:

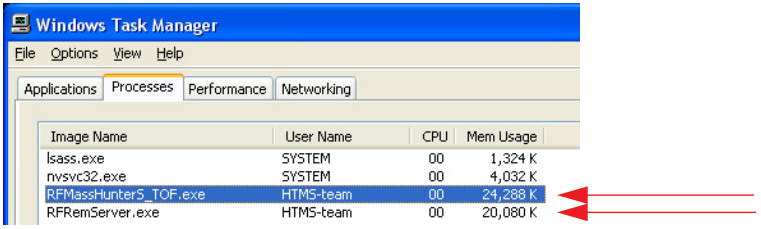
- Confirm that MassHunter Data Acquisition program is installed on the computer.

1 Click **RapidFire Communicator** on the Windows Start menu or desktop.

The following program files are started:

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

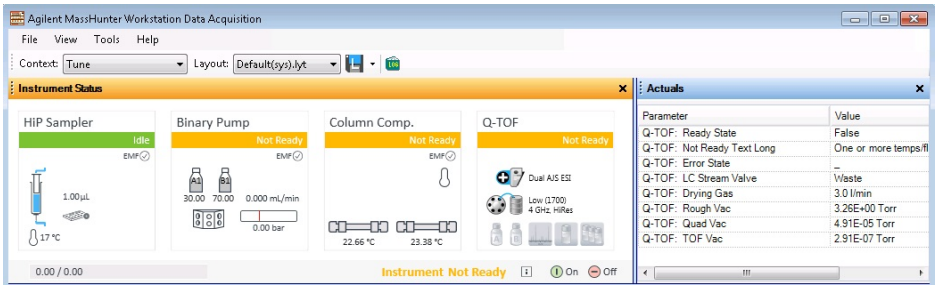
These program files are required for the software integration of the RapidFire System.



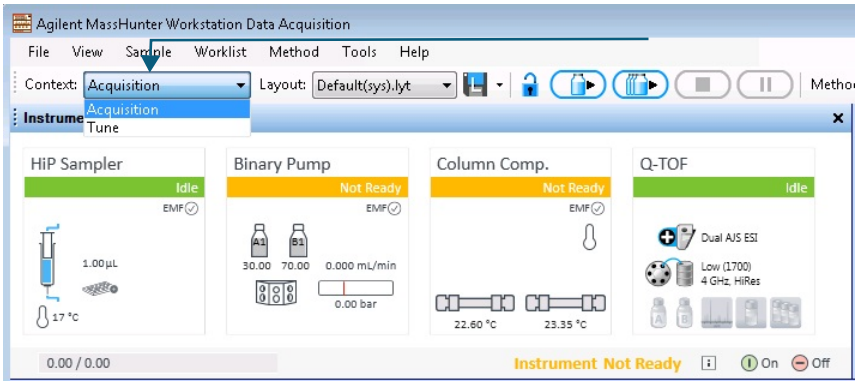
2 To start the MassHunter Data Acquisition software, double-click the **Data Acquisition** desktop icon.

3 Turn on the detector.

The title of the device in the Instrument Status window turns from yellow (Not Ready) or teal (Standby) to green (Idle).



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



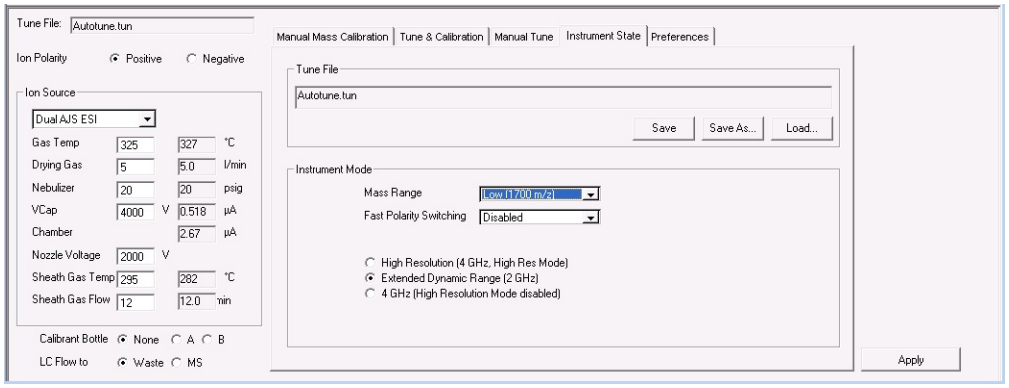
5 Set the mass and resolution parameters for your analysis in the Instrument State tab in the Tune window.

Tip

- High Resolution (scan speed 4 GHz)
- Extended Dynamic Range (scan speed 2 GHz)
- Minimum Storage Size (scan speed 1 GHz)

a Click **Apply** on the right.

b If necessary, wait for the system to equilibrate.



6 Calibrate the mass spectrometer.

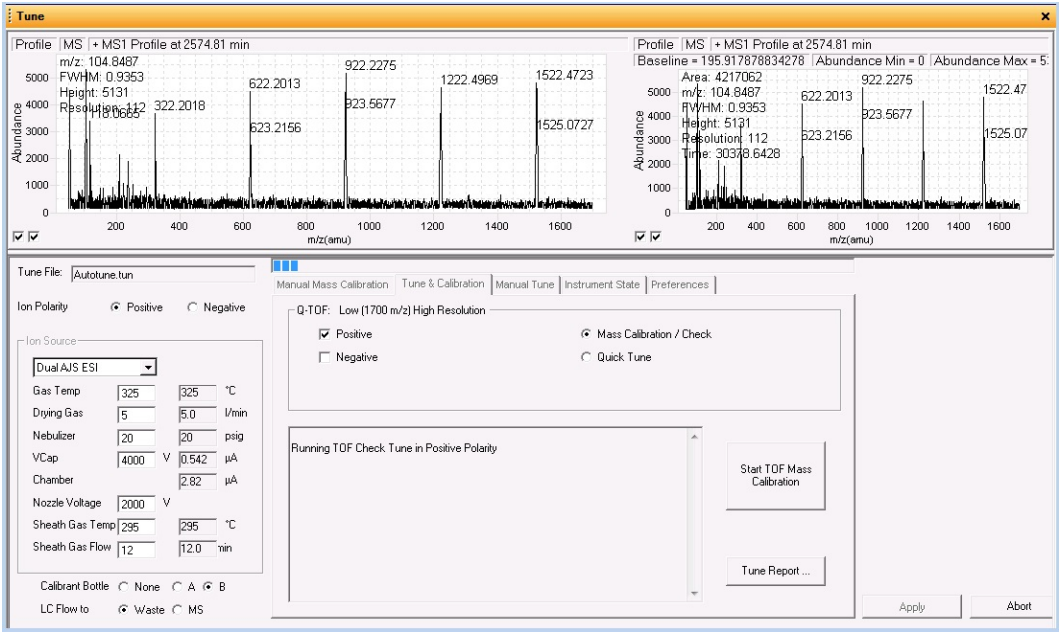
a Set the location of the Calibrant Bottle (example: B), and wait for the mass-over-charge spectrum to stabilize.

b Click the **Tune & Calibration** tab.

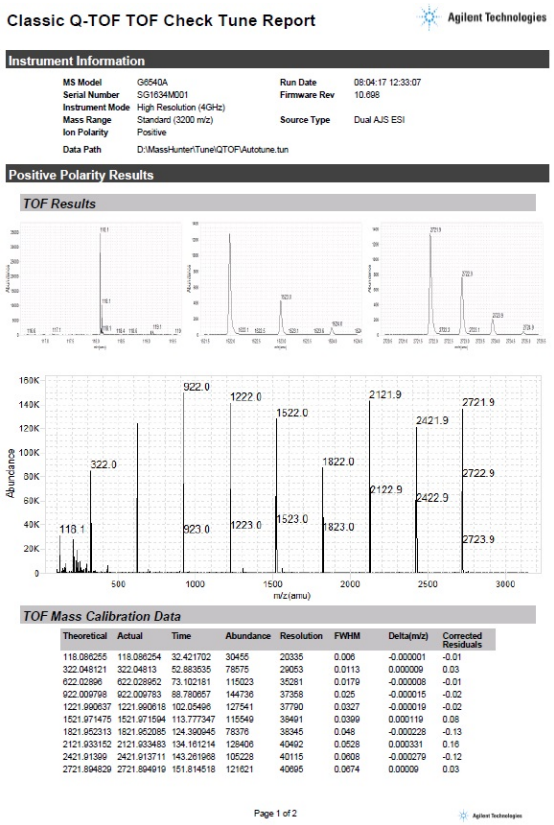
c Mark the **Polarity** to use. You can mark both check boxes.

d Click **Mass Calibration / Check**.

e Click **Start TOF Mass Calibration**.



f When the Check Tune Report appears, check the results. For example, check that the calculated residual errors are satisfactory (for example < 2.0 ppm).



Classic Q-TOF TOF Check Tune Report

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Instrument Information

MS Model	G6540A	Run Date	08:04:17 12:33:07
Serial Number	SG1634M001	Firmware Rev	10.698
Instrument Mode	High Resolution (4GHz)		
Mass Range	Standard (3200 m/z)	Source Type	Dual AJS ESI
Ion Polarity	Positive		
Data Path	D:\MassHunter\Tune\QTOF\Autotune.tun		

Positive Polarity Results

TOF Results

TOF Mass Calibration Data

Theoretical	Actual	Time	Abundance	Resolution	FWHM	Delta(m/z)	Corrected Residuals
118.086255	118.086254	32.421702	30455	20335	0.006	-0.000001	-0.01
322.048121	322.04813	52.883535	78575	29053	0.0113	0.000009	0.03
622.02896	622.028952	73.102181	115023	35281	0.0179	-0.000008	-0.01
922.009798	922.009783	88.780657	144736	37358	0.025	-0.000015	-0.02
1221.990637	1221.990618	102.05496	127541	37790	0.0327	-0.000019	-0.02
1521.971475	1521.971594	113.777347	115549	39491	0.0399	0.000119	0.08
1821.952313	1821.952085	124.390945	78376	38345	0.048	-0.000228	-0.13
2121.933152	2121.933483	134.161214	126406	40492	0.0528	0.000331	0.16
2421.91399	2421.913711	143.261968	105228	40115	0.0608	-0.000279	-0.12
2721.894829	2721.894919	151.814518	121621	40695	0.0674	0.00009	0.03

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

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

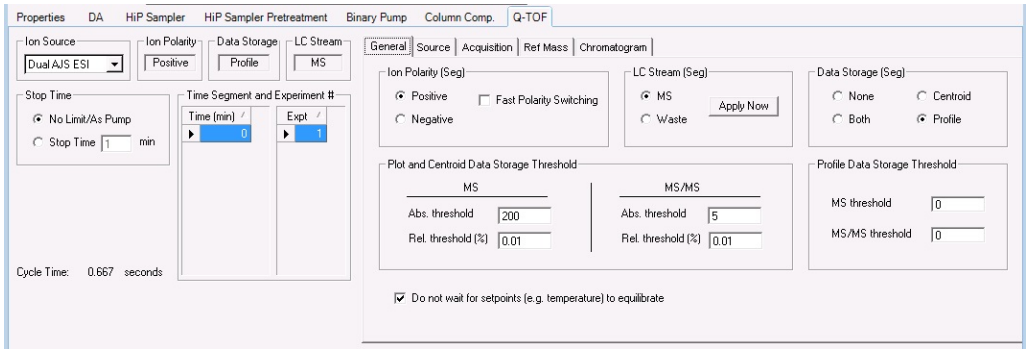
7 Select **Acquisition** in the Context list box.

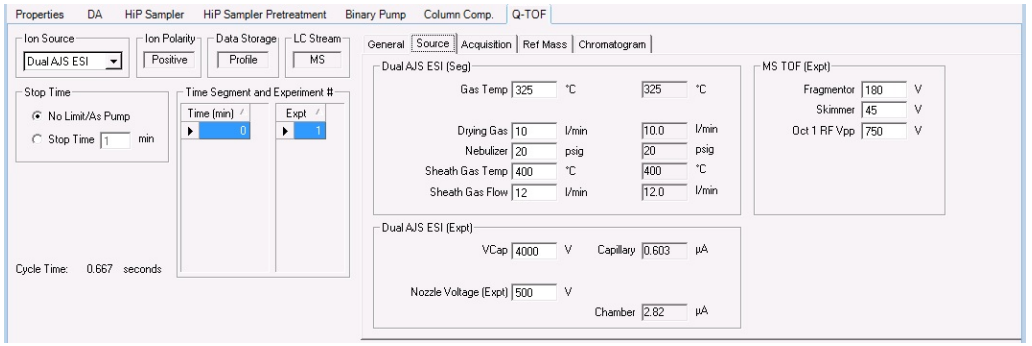
If prompted, confirm that you want to operate under the modified Instrument State. ***DO NOT modify the Tune File.***

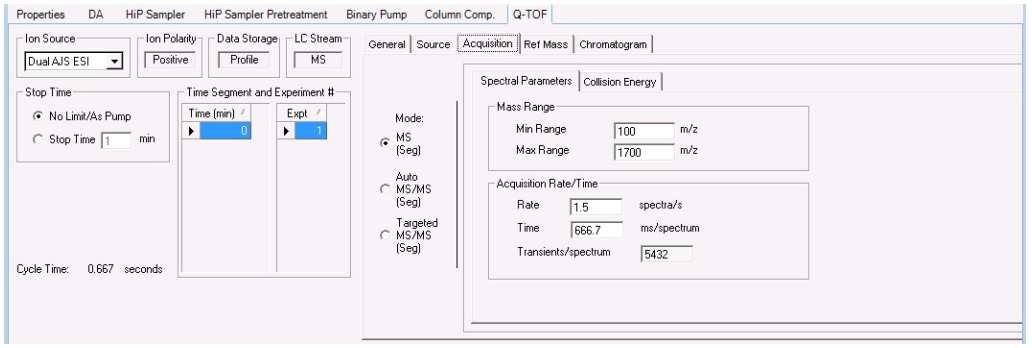
8 Click **Method > Open** to open a **.m** method or set parameters to create a method.

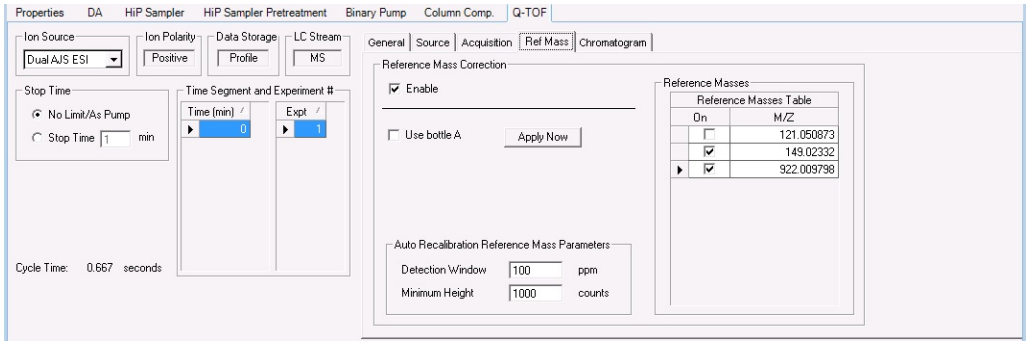
Methods for the RapidFire System are stored in the **D:\MassHunter\methods\RapidFire** folder.

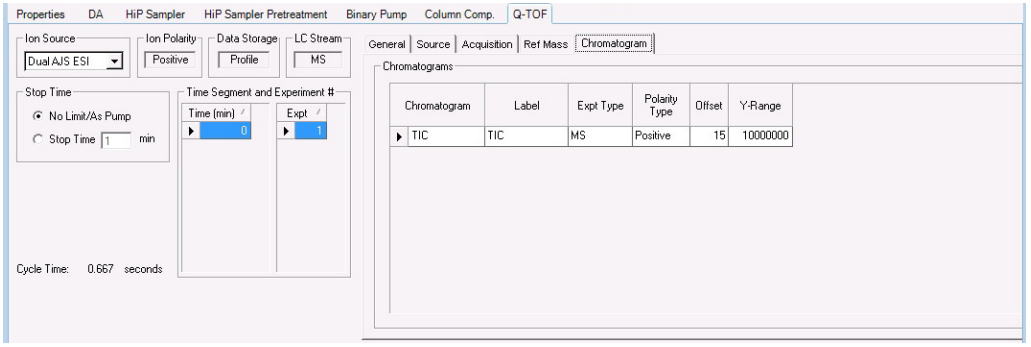
9 Set the parameters for your analysis on the **Q-TOF** tabs shown in the following example screens.


Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump Column Comp. Q-TOF
Ion Source
Dual AJS ESI
Ion Polarity
Positive
Data Storage
Profile
LC Stream
MS
Stop Time
No Limit/As Pump
Stop Time 1 min
Time Segment and Experiment #
Time (min) 0
Expt 1
Cycle Time: 0.667 seconds
General Source Acquisition Ref Mass Chromatogram
Ion Polarity (Seg)
Positive
Negative
Fast Polarity Switching
LC Stream (Seg)
MS
Waste
Apply Now
Data Storage (Seg)
None
Centroid
Both
Profile
Plot and Centroid Data Storage Threshold
MS
Abs. threshold 200
Rel. threshold (%) 0.01
MS/MS
Abs. threshold 5
Rel. threshold (%) 0.01
Profile Data Storage Threshold
MS threshold 0
MS/MS threshold 0
Do not wait for setpoints (e.g. temperature) to equilibrate

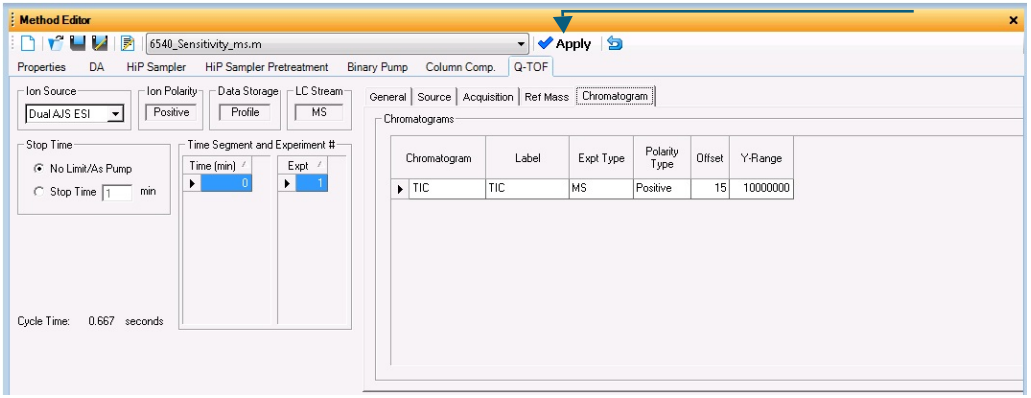

Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump Column Comp. Q-TOF
Ion Source
Dual AJS ESI
Ion Polarity
Positive
Data Storage
Profile
LC Stream
MS
Stop Time
No Limit/As Pump
Stop Time 1 min
Time Segment and Experiment #
Time (min) 0
Expt 1
Cycle Time: 0.667 seconds
General Source Acquisition Ref Mass Chromatogram
Dual AJS ESI (Seg)
Gas Temp 325 °C 325 °C
Drying Gas 10 l/min 10.0 l/min
Nebulizer 20 psig 20 psig
Sheath Gas Temp 400 °C 400 °C
Sheath Gas Flow 12 l/min 12.0 l/min
MS TOF (Expt)
Fragmentor 180 V
Skimmer 45 V
Oct 1 RF Vpp 750 V
Dual AJS ESI (Expt)
VCap 4000 V
Capillary 0.603 µA
Nozzle Voltage (Expt) 500 V
Chamber 2.82 µA


Properties DA HiP Sampler HiP Sampler Pretreatment Binary Pump Column Comp. Q-TOF
Ion Source
Dual AJS ESI
Ion Polarity
Positive
Data Storage
Profile
LC Stream
MS
Stop Time
No Limit/As Pump
Stop Time 1 min
Time Segment and Experiment #
Time (min) 0
Expt 1
Cycle Time: 0.667 seconds
General Source Acquisition Ref Mass Chromatogram
Spectral Parameters Collision Energy
Mode:
MS (Seg)
Auto MS/MS (Seg)
Targeted MS/MS (Seg)
Mass Range
Min Range 100 m/z
Max Range 1700 m/z
Acquisition Rate/Time
Rate 1.5 spectra/s
Time 666.7 ms/spectrum
Transients/spectrum 5432



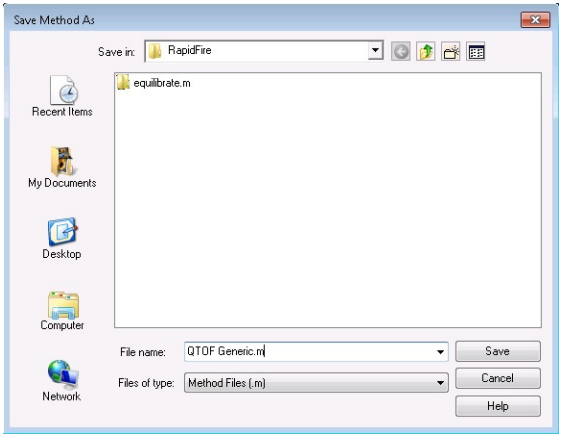


10 When you have finished setting the parameters, click **Apply** in the toolbar for the Method Editor window.



11 Save the method.

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



To prepare the MassHunter Workstation for Triple Quad LC/MS

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:

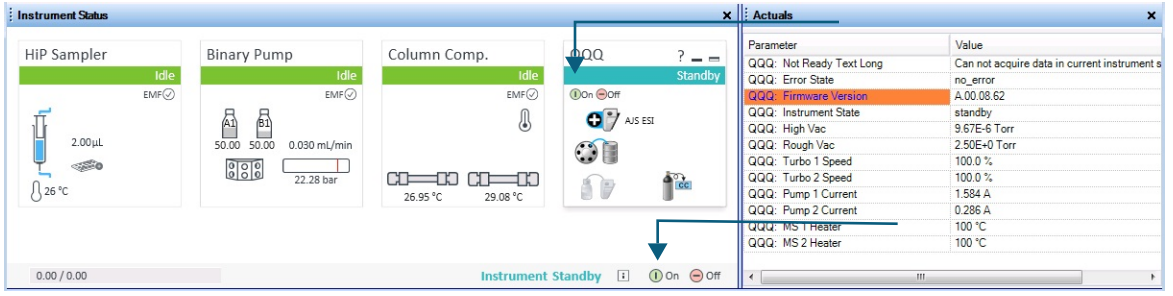
- **RFRemServer.exe**
- **RFMassunterS_QqQ.exe**

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**. You can click **On** in the QQQ device pane or in the status line for the Instrument Status window.



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

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 24.
   b. Clean the fluidics lines by running a few rows of methanol wells. A minimum of 50 wells is recommended.
2. 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.
3. 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.
4. Turn off the vacuum source.

2 Maintenance

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

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 24.
2. Do the shutdown procedures described in "To turn off the system at the end of the day" on page 34.

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

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

To prepare plates

1 Completely thaw the sample plates.

2 Centrifuge the plates at 3000 rpm for at least 5 minutes.

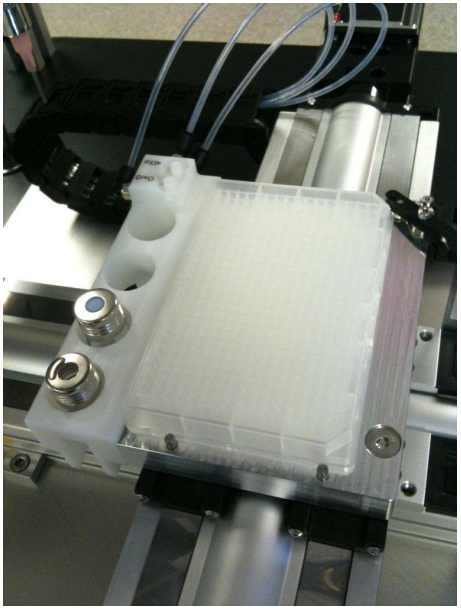
Tip Spin down the plates, particularly if samples contain insoluble particulates, such as microsomes. When you spin the plates, particles and insoluable material are forced to the bottom of the wells where they are less likely to be aspirated into the system.

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 15.

2 Securely position the plate on the holder in between the metal pins, and then close the front doors.



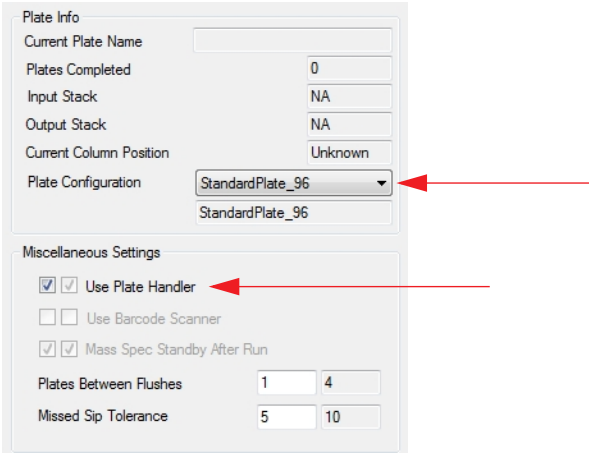
To load plates using the BenchBot plate handler

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 Select **Use Plate Handler**. Click **Apply** to register the change.



2 Stack the plates to be analyzed in the input plate stackers.

NOTE Leave at least one plate stacker empty to be used as the initial output plate stacker.

To prepare the MassHunter Workstation for TOF or Q-TOF LC/MS

1 Set up the mass spectrometer computer and turn on the mass spectrometer as described in "To prepare the MassHunter Workstation for TOF or Q-TOF LC/MS" on page 25.

2 On the Sample Run window 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).

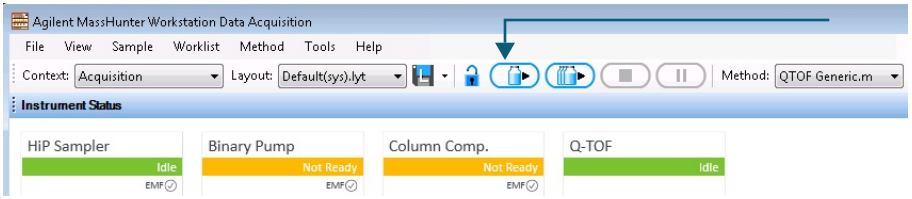
   b Click  to add a parameter to the **Additional Information** list.

   c Select **RunType** and click **OK**.

   d For the **Run Type**, select **Manual Run**.

   e Set the **Injection Volume** to **As Instrument**. This value causes the system to use the injection volume set by the RapidFire injection loop (at V1P2-V1P5).

3 Click the **Start Sample Run** button, or click **Sample > Run**.

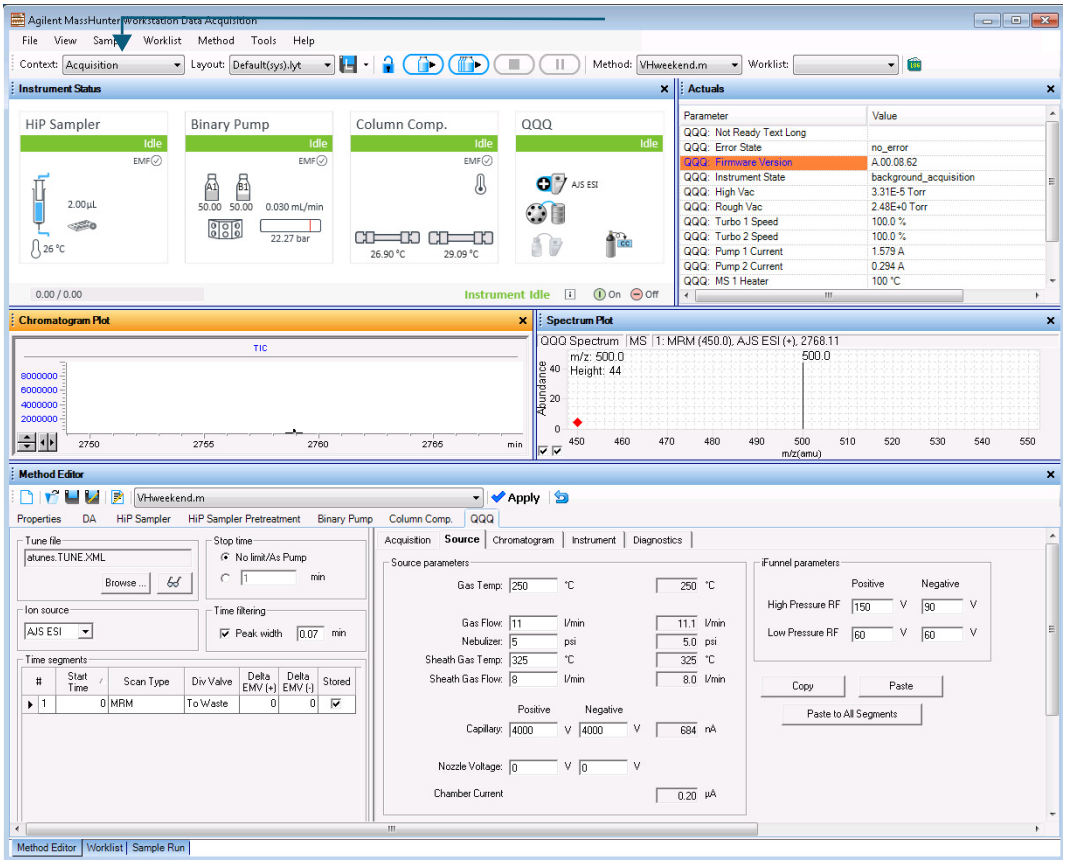


When the source has equilibrated, the label of the Q-TOF device module turns blue in the Instrument Status window.

To prepare the MassHunter Workstation for Triple Quad LC/MS

1 Set up the mass spectrometer computer and turn on the mass spectrometer as described in "To prepare the MassHunter Workstation for Triple Quad LC/MS" on page 33.

2 Select **Acquisition** in the Context list.



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

Agilent recommends to store methods used for the RapidFire-MS System in the **D:\MassHunter\methods\RapidFire** folder.

4 Set the parameters for your analysis on the **QQQ** tabs.

5 On the **Sample Run** window 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).

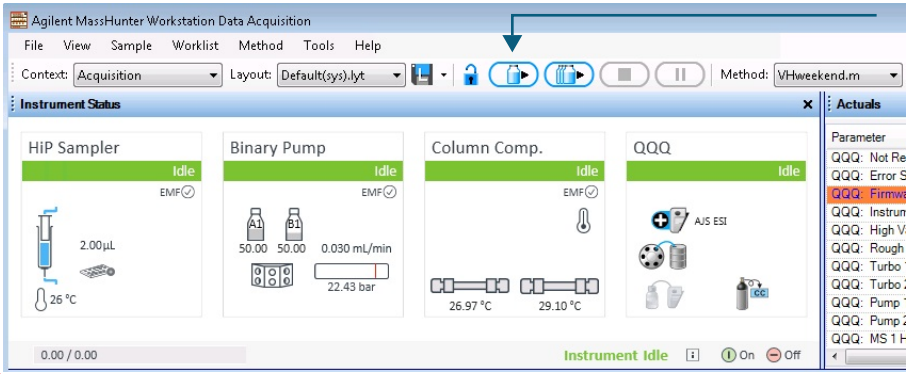
b Click  to add a parameter to the **Additional Information** list.

c Select **RunType** and click **OK**.

d For the **Run Type**, select **Manual Run**.

e Set the **Injection Volume** to **As Instrument**. This value causes the system to use the injection volume set by the RapidFire injection loop (at V1P2-V1P5).

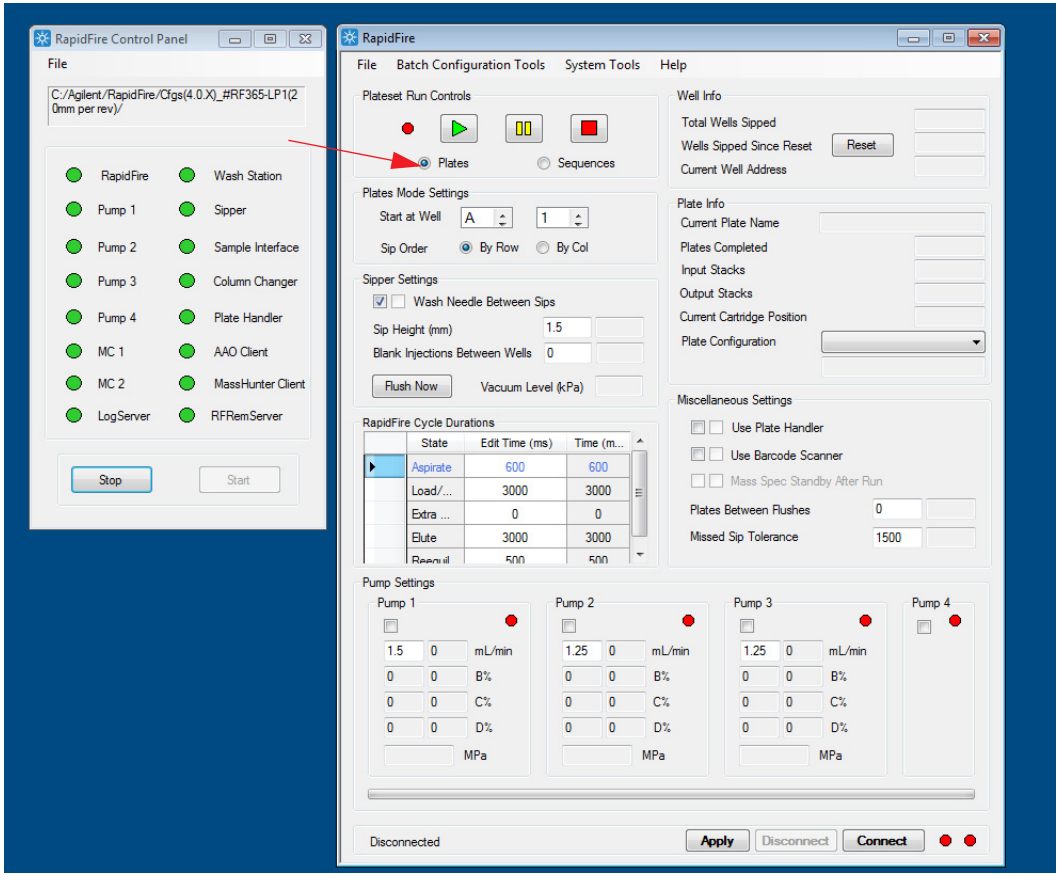
6 Click the **Start Sample Run** button, or click **Sample > Run**.



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

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.



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, and 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

Runs in Plates mode are *not* synchronized with the mass spectrometer. Initiate the mass spectrometer acquisition run *before* RapidFire starts.

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, and 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.

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, and 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, and 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, and then the RapidFire System stops operation.
- Data acquisition is stopped and the current data file is closed.

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 49, *or*

"To stop a run" on page 49.

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

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

To prepare plates

1 Completely thaw the sample plates.

2 Centrifuge the plates at 3000 rpm for at least 5 minutes.

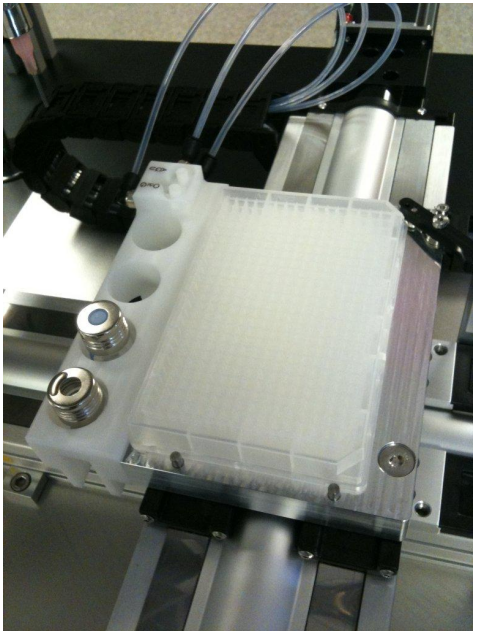
Tip Spin down the plates, particularly if samples contain insoluble particulates, such as microsomes. When you spin the plates, particulates are forced to the bottom of the wells, where it is less likely to be aspirated into the system.

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 15.

2 Securely position the plate on the holder in between the metal pins, and then close the front doors.

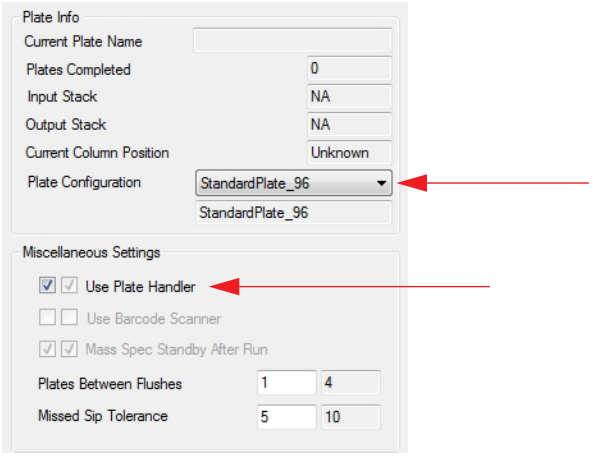


To load plates using the BenchBot plate handler

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

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.

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



2 Stack the plates to be analyzed in the input plate stackers, as shown in the next figure.

NOTE Leave at least one plate stacker empty to be used as the initial output plate stacker.

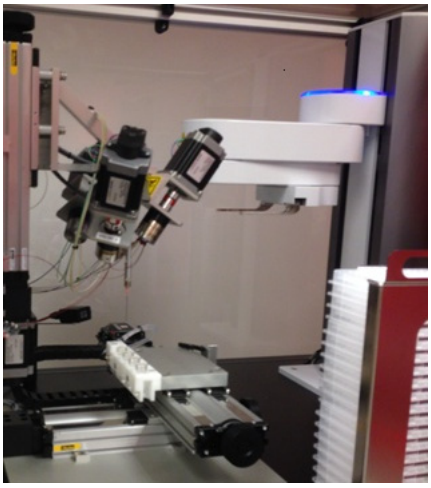
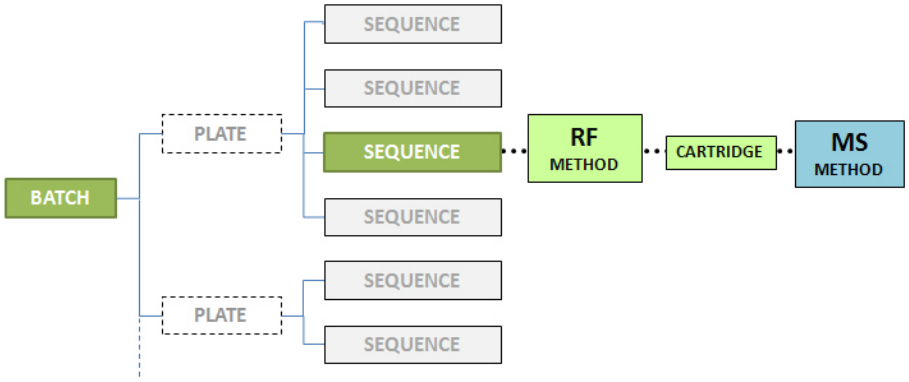


Figure 1 Plates stacked in an input plate stacker

To run plates

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

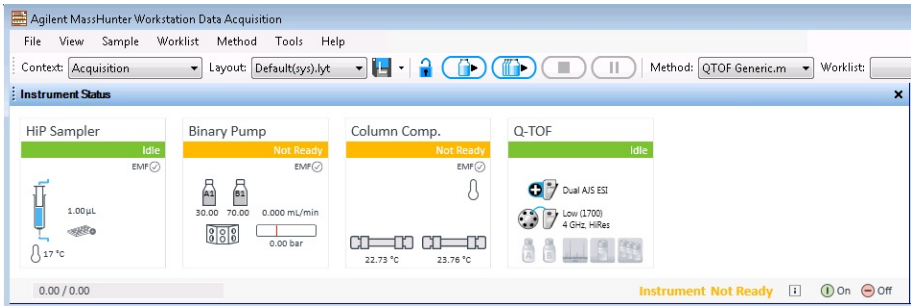


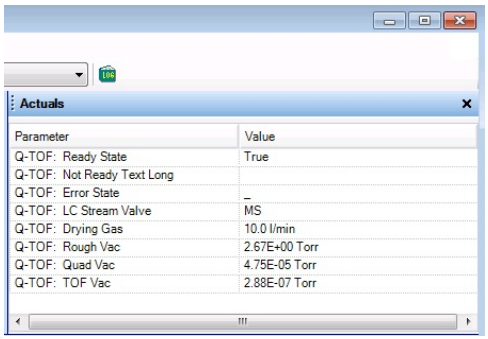
To prepare the mass spectrometer computer

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

- "To prepare the MassHunter Workstation for TOF or Q-TOF LC/MS" on page 25

  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.

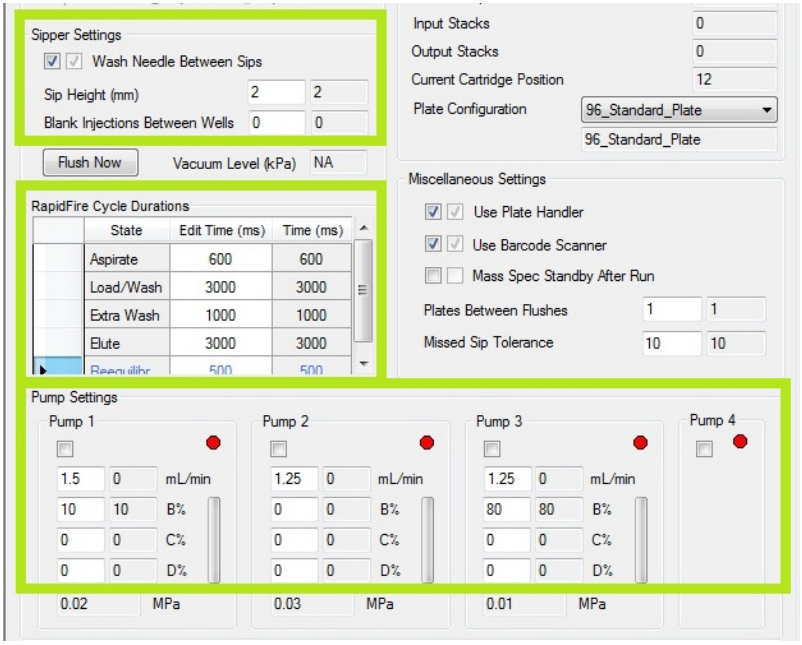
- "To prepare the MassHunter Workstation for Triple Quad LC/MS" on page 43

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

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.



2 In the **Sipper Settings** area of the screen:

a Select **Wash Needle Between Sips** to wash the sipper tube once after aspiration of the sample from each well. The tube is washed first in the aqueous, and 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.

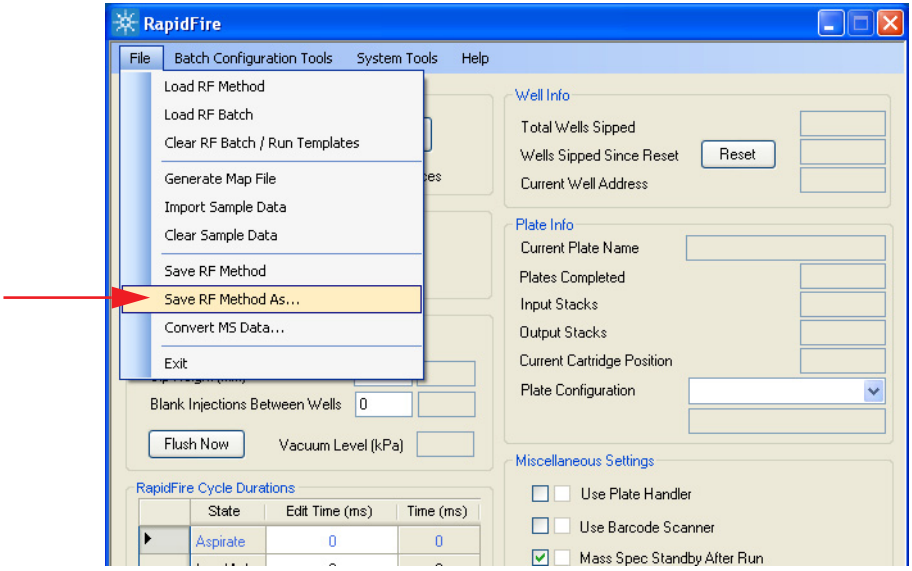
3 In the **RapidFire Cycle Durations** area, set the valve timing, as per assay protocol for States 1 - 5 (**Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, and **Re-equilibrate**).

- 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.

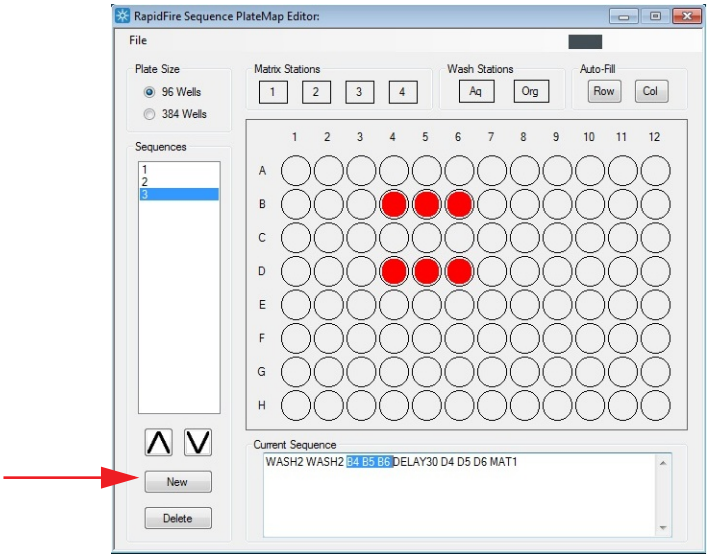


To edit the plate map

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, and then proceeds with the next sequence listed in the Batch Editor.

To edit the batch

CAUTION Any changes you make to a .rfmap or .rfcfg file after the file is used to create or edit a batch is disregarded by the batch. For example, if you create a batch using an .rfmap file, then you edit the .rfmap file to include an additional well, the additional well will not be used when you run 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 61.

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**.

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**).

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, sse **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**.

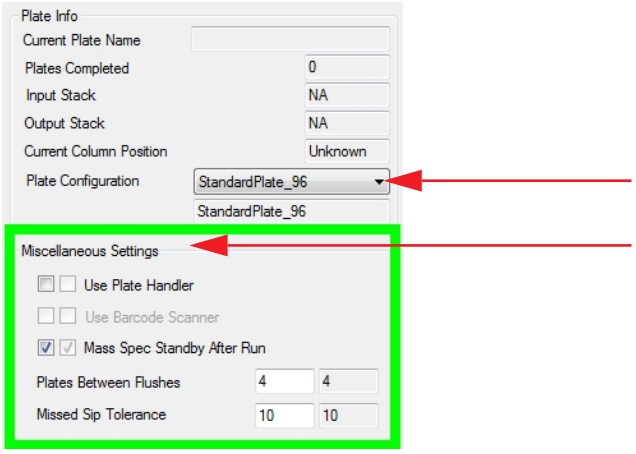
b Select the batch of interest (**.rfbat** file).

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.



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 Select whether to:

- **Use Plate Handler**
- **Use Barcode Scanner**
- For Triple Quad, put the **Mass Spec on Standby After the Run**.
- For Q-TOF, *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**.

NOTE In the next step, if you selected **Use plate handler** and **Use barcode scanner**, disregard step a and step b. If the robot and bar code scanners are used, all of the plates in the plate stacker will be serviced in the order that they appear in the stacker. Each plate will be serviced as specified in the batch file if its barcode appears in the batch file.

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 54.
- 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.

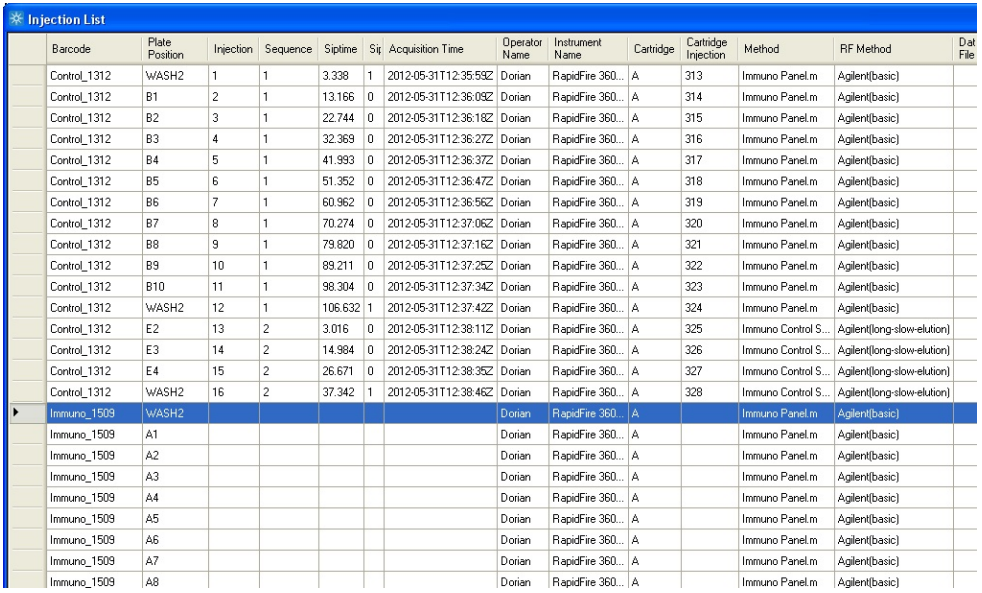
4 To monitor a run, use the following features:

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).

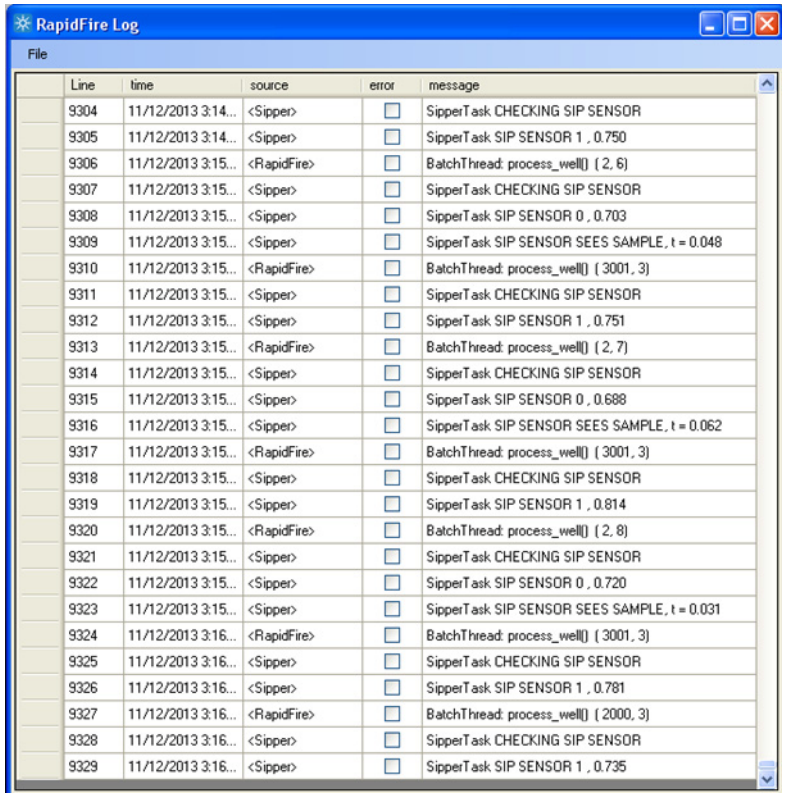
b To monitor the progress of your RapidFire run, click **System Tools > View Injection List** as shown in the following example:



Injection List

Barcode	Plate Position	Injection	Sequence	Siptime	Sip	Acquisition Time	Operator Name	Instrument Name	Cartridge	Cartridge Injection	Method	RF Method	Dat File
Control_1312	WASH2	1	1	3.338	1	2012-05-31T12:35:59Z	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)	

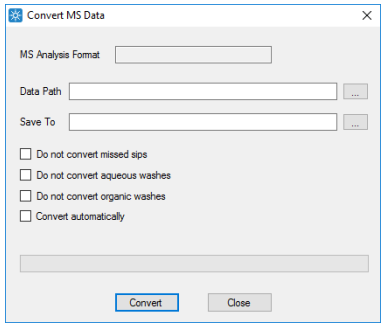
c Click **System Tools > View Log** to monitor the progress of your RapidFire run as shown in the following example:



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:

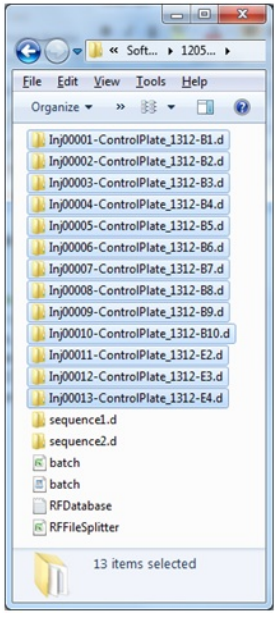
- Click **File > Convert MS Data**.



- 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.
- If needed, select options to indicate what you you do not want to be converted.
- Select **Convert automatically** if you want data to be converted automatically.

  While this option is selected and the dialog box opened, data is automatically converted. **Data Path** and **Save to** are automatically populated with the data folder that contains data most recently imported or generated by a run. Conversion begins on the data in that directory immediately. When you clear the check box, automatic conversion in progress is canceled, and **Data Path** and **Save to** are no longer automatically populated.
- Click **Convert**.

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:



- To analyze the data, refer to MassHunter Workstation Quantitative Analysis user guides 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, and 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, and then the RapidFire System stops operation.
- Data acquisition is stopped and the current data file is closed.

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 69, *or*

"To stop a run" on page 69.

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 Reference

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

Specifications

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 75.

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

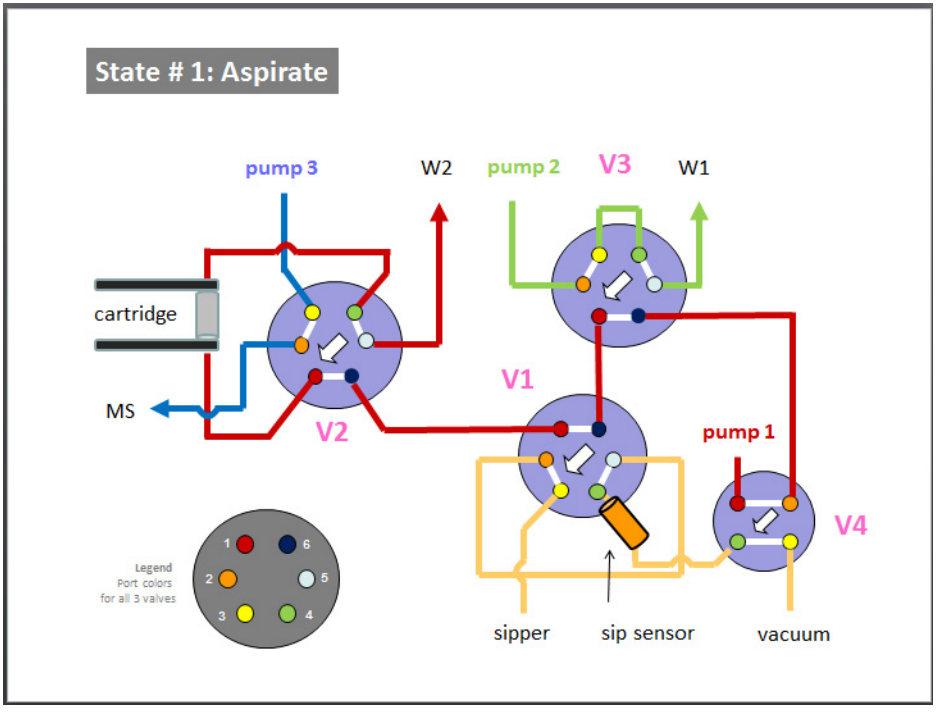
- 1 liter graduated cylinder
- Pipettes and pipette tips
- Sample 96- and 384-well plates with standard bar codes

RapidFire System Cartridges

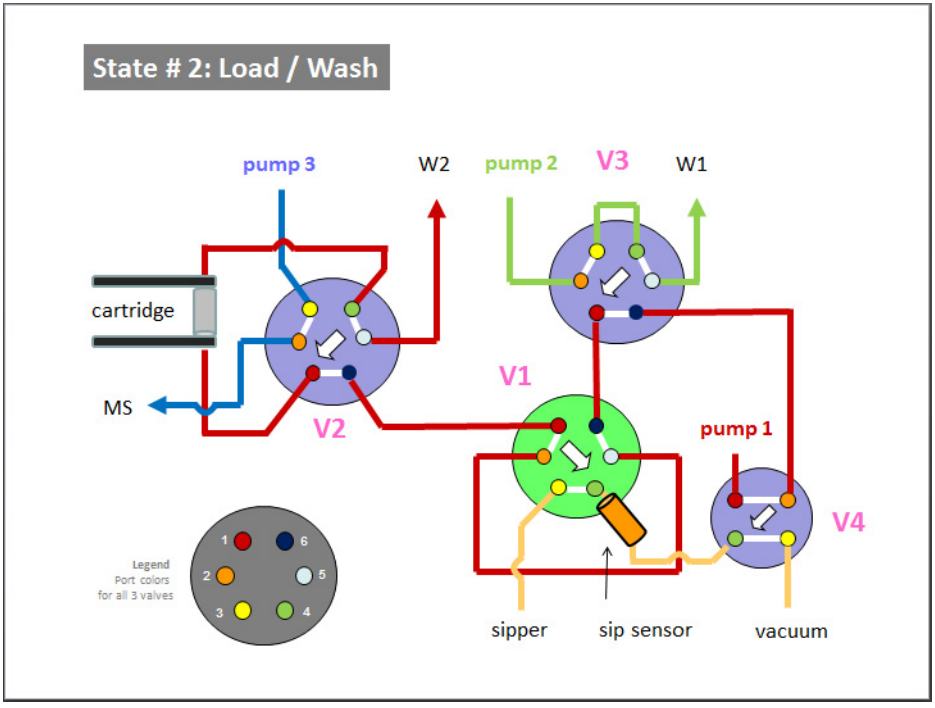
Part number	Type	Packing material
G9203A	A	C4
G9204A	B	Cyano
G9205A	C	C18
G9206A	D	Graphitic carbon
G9207A	E	C8
G9208A	F	Phenyl
G9209A	H1	HILIC
G9210A		Custom
G9211A	0	Blank
G9525A	A2	C4

RapidFire System Flow Path

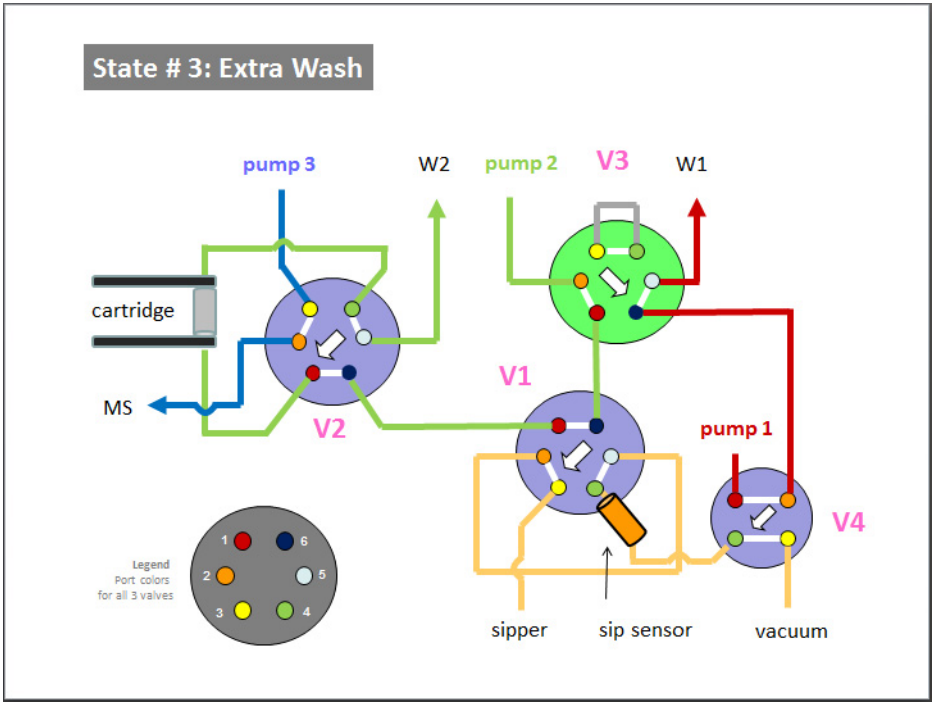
State #1: Aspirate



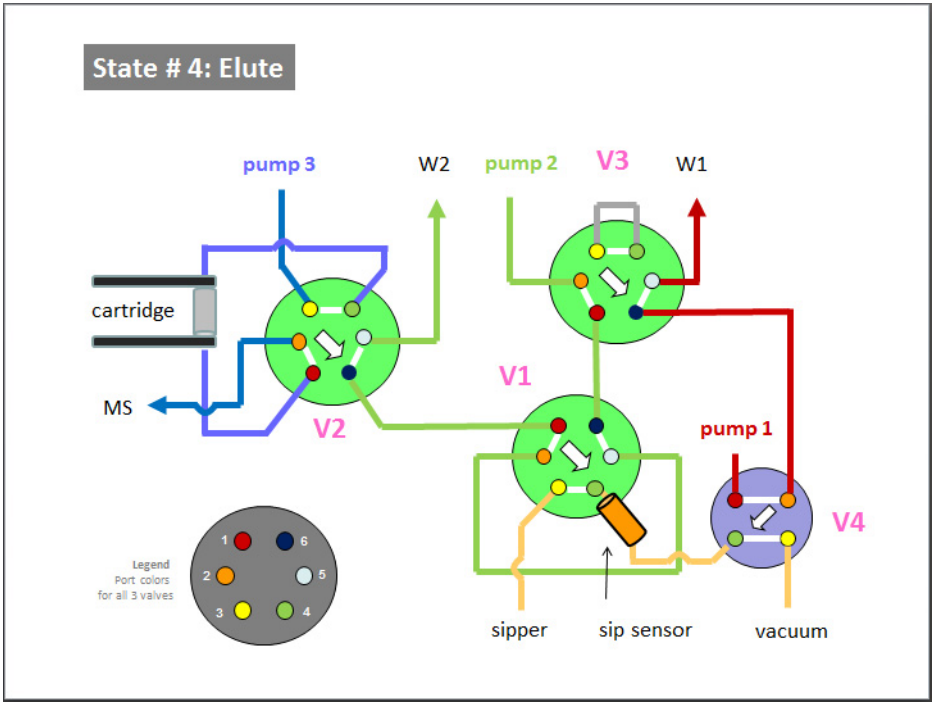
State #2: Wash/Load



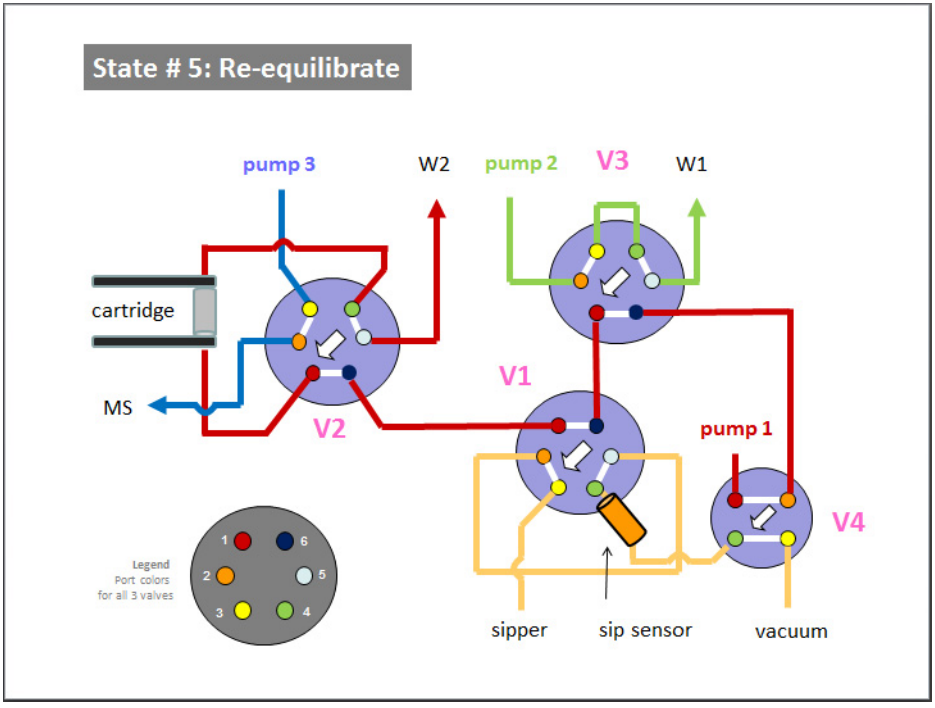
State #3: Extra Wash



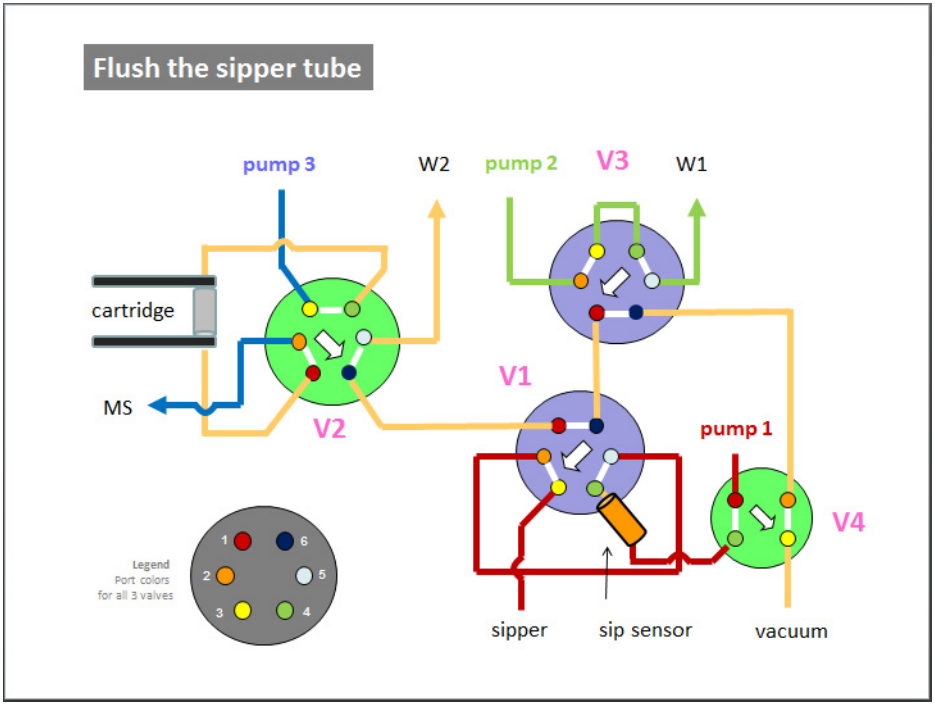
State #4: Elute



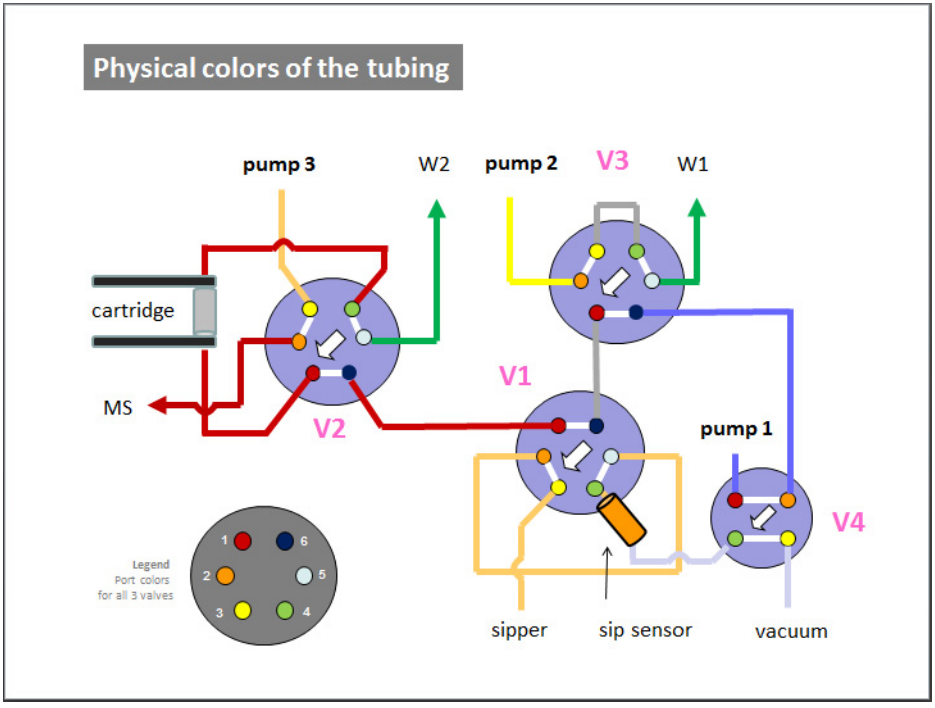
State #5: Re-equilibrate



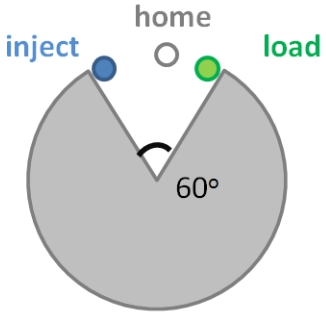
Flush the sipper tube



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.

For Research Use Only. Not for use in diagnostic procedures.

Instrument Manufacturing

Manufactured by Agilent Technologies Singapore Pte. Ltd. No. 1 Yishun Avenue 7, Singapore 768923

Operating Temperature: 4°C to 35°C

Revision A, August 2017

G9531-90003

Agilent Technologies