Notices

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Safety Notices

CAUTION

A CAUTION 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 damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

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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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.

![Image of RapidFire Control Panel with icons turning green]

The icons turn green as the system components start up.

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

![Image of RapidFire UI showing 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.
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.

- Manually adjust the stage to clear the plate, wash station, and matrix bottles by about 0.5 cm or 1/4”.
- 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.
To check the valves

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

![Image of Valve Configuration Utility](image)

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:

<table>
<thead>
<tr>
<th>Valve position</th>
<th>Pump number</th>
<th>Back pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1, V2, and V3 blue (Inject position)</td>
<td>P1</td>
<td>~1 - 10 MPa (or 10 - 100 bar)</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>~0.1 - 3 MPa</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>~1 - 10 MPa</td>
</tr>
<tr>
<td>V1, V2, and V3 green (Load position)</td>
<td>P1</td>
<td>~0.1 - 10 MPa (lower than in blue or Inject)</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>~0.1 - 3 MPa (higher than in blue or Inject)</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>~1 - 10 MPa (higher than in blue or Inject)</td>
</tr>
</tbody>
</table>

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.

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).
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.

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

---

**Table 1: Parameters for System Preparation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Properties</th>
<th>MS Q-TOF</th>
<th>Data Storage</th>
<th>LC Stream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Source</td>
<td></td>
<td>ESI</td>
<td>Positive</td>
<td>Profile</td>
</tr>
<tr>
<td>Agilent Lab Solutions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop Time</td>
<td></td>
<td>No Limit/Az Pump</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle Time</td>
<td>0.2 s</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Figure 1: System Configuration**

The image shows a screenshot of the Agilent MassHunter Q-TOF LC/MS computer setup, with various parameters and settings highlighted. The configuration includes settings for ion source, stop time, and cycle time, among others.

---

**Figure 2: Additional System Parameters**

The additional parameters include settings for data storage, LC stream, and general acquisition settings. The image also shows a fragment ion table and acquisition parameters.

---

**Figure 3: TOF Spectra**

The TOF spectra details include mass range, acquisition rate, and transient spectrum settings. The image provides a visual representation of the TOF spectra with relevant parameters highlighted.
10 When you have finished setting the parameters, click **Apply** on the right side of the screen.
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.

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.
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 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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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 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.

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).

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).

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.
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-1,25mLtune.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**.
3 Operation in Plates Mode
To start a run in Plates mode

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.

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.

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

![E-STOP button](image)

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.
4 Operation in Sequences Mode

To prepare plates 58
To load plates manually 58
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To run plates 61
To prepare the mass spectrometer computer 62
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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 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:
   - Select the plate configuration (96- or 384-well).
   - Mark the **Use Plate Handler** option, then click **Apply**.

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.

[Diagram showing 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.
4 Operation in Sequences Mode

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 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 \texttt{DELAY\#}, where \# is an integer that specifies the number of seconds of delay.

\textbf{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.

\section*{To edit the batch}

1. Click \texttt{Batch Configuration Tools > Batch Editor}.
2. To create a batch, click \texttt{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 \texttt{OK}.

\textbf{Tip} If you click \texttt{Cancel} without entering a plate name, then the plate is named Plate1, Plate2, Plate3, and so on, by default.
4. Select the \textbf{MS Integration} software that the RapidFire System run is to be synchronized with, such as \textbf{MassHunter QQQ}.
   a. If you select Analyst, also enter or select a Project to use.
   b. \textit{Integration with Thermo Fisher MS is not currently supported.}
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.

![RapidFire window](image)

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.
4  **Operation in Sequences Mode**
To run a batch in Sequences mode

**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.

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:

   **NOTE** Close the doors while operating the RapidFire System to prevent possible injury. The system does not operate with the doors open.
To run a batch in Sequences mode

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

For data analysis by the MassHunter Workstation Quantitative Analysis software, create individual peak data files as follows:

- Click File > Convert MS Data.
To run a batch in Sequences mode:

- 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:

![Convert MS Data Dialog](image)

- 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.
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.
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

<table>
<thead>
<tr>
<th>Part number</th>
<th>Type</th>
<th>Packing material</th>
</tr>
</thead>
<tbody>
<tr>
<td>G9203A</td>
<td>A</td>
<td>C4</td>
</tr>
<tr>
<td>G9204A</td>
<td>B</td>
<td>Cyano</td>
</tr>
<tr>
<td>G9205A</td>
<td>C</td>
<td>C18</td>
</tr>
<tr>
<td>G9206A</td>
<td>D</td>
<td>Graphitic carbon</td>
</tr>
<tr>
<td>G9207A</td>
<td>E</td>
<td>C8</td>
</tr>
<tr>
<td>G9208A</td>
<td>F</td>
<td>Phenyl</td>
</tr>
<tr>
<td>G9209A</td>
<td>H1</td>
<td>HILIC</td>
</tr>
<tr>
<td>G9210A</td>
<td>Custom</td>
<td></td>
</tr>
<tr>
<td>G9211A</td>
<td>0</td>
<td>Blank</td>
</tr>
<tr>
<td>G9525A</td>
<td>A2</td>
<td>C4</td>
</tr>
</tbody>
</table>
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

[Diagram of RapidFire System Flow Path]
State #2: Wash/Load
State #3: Extra Wash
State #4: Elute
5 Technical Specifications and Parts

State #5: Re-equilibrate

State #5: Re-equilibrate
Flush the sipper tube
5 Technical Specifications and Parts

Physical colors of the tubing

Valve positions
In This Book

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