Agilent

RapidFire 400 System

User Guide

Original instructions

Notices

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Software Revision

This guide is valid for the C.01.00 revision or higher of the PRODUCT NAME program and compatible PRODUCT NAME programs, until superseded.

Instrument Manufacturing

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

Temperature Limits

Operating Temperature: 15°C to 35°C
Storage Temperature: -40°C to 70°C

Software Manufacturing

Manufactured for Agilent Technologies
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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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1 Before you start

This section provides an overview of the RapidFire 400 System, where to find more information, safety notes, and materials supplied. It contains the following topics:

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About this guide

Who should read this guide

This guide is for people with the following job roles:

Table 1. Job roles and responsibilities

Job role	Responsibilities
Installer	Unpacks, installs, and tests the device before it is used.
Integrator	Configures hardware and writes software.
Lab manager, administrator, or technician	• Manages the automation system that contains the device. • Develops the applications that are run on the system. • Develops training materials and standard operating procedures for operators.
Operator	Performs the daily production work on the device and solves routine problems.

What this guide covers

This guide covers the description, setup, operation, and maintenance of the Agilent G9532A and G9532AA RapidFire 400 System.

This guide does not provide instructions for unpacking, installation, and data analysis.

What is new in this guide

Table 2. What is new in this guide

Change	Described in...
Release of MassHunter 11.0 (MH11) for TOF/QTOF. MH11 uses components of the OpenLab software which affect certain procedures and the user interface.	• **"Preparing the TOF or Q-TOF mass spectrometer"** on page 53. • **"Preparing the TOF or Q-TOF mass spectrometer"** on page 70. • **"Preparing the TOF or Q-TOF mass spectrometer"** on page 97
New default sequence of sip operation	**"Changing the sequence of the sipper operation"** on page 125

Software version and compatibility

This guide documents RapidFire Acquisition software v 6.0.

RapidFire Acquisition software 6.0 requires the Microsoft Windows 10 64-bit operating system.

RapidFire Acquisition software 6.0 is compatible with:

- MassHunter Acquisition software 10.1 for TOF, Q-TOF, and TQ
- MassHunter Acquisition software 1.2 for Ultivo

Related guides and more information

Use this guide in conjunction with the following:

- *RapidFire 400 System Safety Guide.* Describes the potential safety hazards and how to avoid them.
- *RapidFire System Data Analysis Guide*. Describes how to process data acquired from the RapidFire System using RapidFire Integrator or MassHunter Quantitative Analysis.
- Agilent web site: **www.agilent.com**.

Safety notes

WARNING

Using controls, making adjustments, or performing procedures other than those specified in the user documentation can expose you to moving-parts hazards and hazardous voltage. Before using the RapidFire 400 System, make sure you are aware of the potential hazards and understand how to avoid being exposed to them.

Ensure you have read the *RapidFire 400 System Safety Guide* and are trained in the safe operation of the device.

Making an emergency stop

CAUTION

Use the emergency stop only for emergencies. If you stop a run with the emergency stop, you cannot continue the run. Otherwise, use one of the following procedures to stop the RapidFire System.

"Pausing a run in Plates mode" on page 79

"Stopping a run in Plates mode" on page 79

Procedure

To stop in an emergency:

1 Press the red emergency stop button on the front of the RapidFire system.

Power is cut to the motors and there is no further motion.

2 To restart the system:

a Release the emergency stop by twisting the knob while applying light pressure.

b Restart the run to continue.

About the RapidFire 400 System

Product description

The RapidFire 400 System is a sample purification and injection system that interfaces directly with the mass spectrometer. The RapidFire System can simultaneously analyze multiple analytes in complex mixtures and remove many contaminants contained in those mixtures.

An optional temperature control unit enables the system to keep plates chilled while stored.

Hardware overview

Primary hardware components

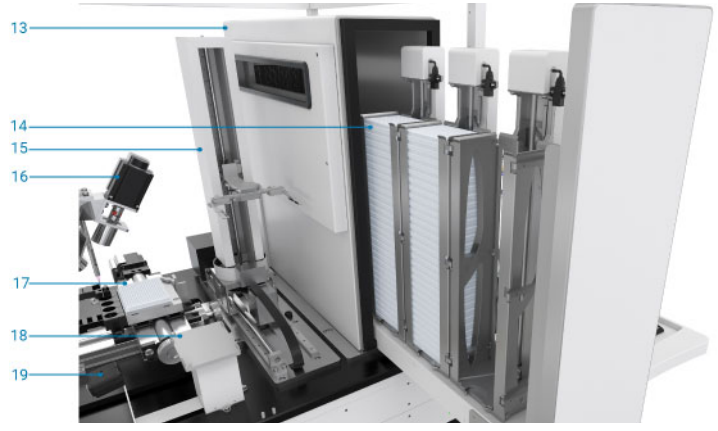

Table 3. RapidFire 400 System components

Item	Name	Description
1	RapidFire computer and monitor	Runs the RapidFire software and mass spectrometer software and communicates to the hardware. The monitor displays the user interface of the controlling and acquiring software.
2	Controller	Controls the chiller, if present, robot, and barcode reader stage.
3	Controller	Controls the plate stage, cartridge changer, and valves.
4	Ultivo Triple Quadrupole LC/MS (not included with the RapidFire 400 System)	Analyzes RapidFire samples.
5	Peristaltic pump	Pumps solvent to sipper wash station.
6	Quaternary pumps	Pump mobile phase throughout the system.
7	Pump with Quiet Cover for Ultivo Triple Quadrupole LC/MS (not included with the RapidFire 400 System)	Provides vacuum for the mass spectrometer.
8	Wheels	Locking wheels to enable system mobility. To lock wheels, step down on the locking tab. To unlock, lift up the locking tab.
9	Solvent and waste secondary container	Holds the waste and liquids that supply the system.
10	Emergency stop button	Stops the motion of the system in an emergency.
11	Doors with safety interlock	Protects you from the moving parts of the system.
12	RapidFire 400 frame	Houses the RapidFire 400 System.
13	Temperature control unit (optional)	Maintains the temperature of sample plates (4° C to ambient)
14	Stacker	Holds the sample plates and moves them into robot-accessible position.
15	Plate handler	Moves the sample plates between the stacks, the barcode stage, and the plate stage.
16	Nanovalves	Direct the flow of mobile phase and samples throughout the system.
17	Plate stage	Controls x, and y-axis movement of plates to facilitate loading and unloading of plates and sipper access to wells.

Table 3. RapidFire 400 System components

Item	Name	Description
18	Barcode scanner stage	Holds the sample plate in position to enable scanning.
19	Barcode scanner	Scans the sample plate.

CAUTION

All interconnecting cables in the RapidFire 400 System must be less than three meters.

Materials supplied

Agilent-supplied items

RapidFire System contents:

- RapidFire 400 System instrument
- *Optional*. Temperature control unit
- Three digitally controlled high-pressure fluidic pumps
- Digital peristaltic pump
- Two barcode scanners
- Waste collection vessels
- 12-slot cartridge changer
- Sip sensor

Consumables:

- Standard RapidFire solid-phase extraction (SPE) cartridges (see the table below)

Customer-supplied items

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

WARNING

Improper handling of solvents can result in personal injury. Handle solvents safely as described in the *Agilent G9532A RapidFire 400 Safety Guide*. Contact the manufacturer for safety data sheets (MSDS) for all materials that you use.

- 1-liter graduated cylinder
- Pipettes and pipette tips
- Sample 96-, 384- and/or 1536-well plates (with or without standard bar codes)

RapidFire system cartridges

There are a number of different types of RapidFire cartridges available from Agilent, some of which are supplied with the instrument. See the RapidFire System ordering catalog for details.

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2 System preparation

This section describes how to prepare the RapidFire 400 System for a run. It contains the following topics:

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Preparing solvents

Use only LCMS-grade, filtered, degassed solvents. Change solvents according to your assay.

WARNING

Improper handling of solvents can result in personal injury. Handle solvents safely as described in the *RapidFire 400 System Safety Guide*. Contact the manufacturer for safety data sheets (MSDS) for all materials that you use.

To prepare the solvents:

1. Check that you have enough solvent for the runs you plan to make and fill the following containers accordingly:
   - Lines 1A, 1B, 1C, 1D feed Pump 1
   - Lines 2A, 2B, 2C, 2D feed Pump 2
   - Lines 3A, 3B, 3C, 3D feed Pump 3
   - Water in a bottle labeled 100% water
   - Acetonitrile in a bottle labeled 100% acetonitrile
   - Other solvents such as methanol, in a labeled bottle
2. Gently shake the filters and whirl the flask around to remove air bubbles.

Checking waste capacity

WARNING

Discard all waste according to your lab's waste disposal procedures and in compliance with all local, state, and national safety regulations.

To check waste capacity:

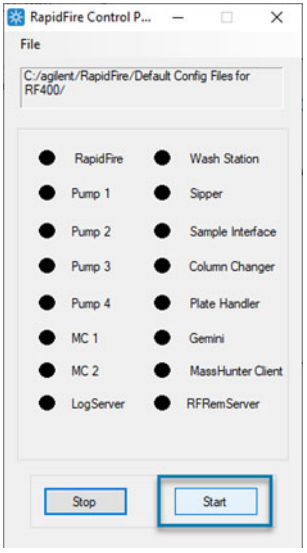
1. Check that the waste containers have enough room to accommodate the waste that will be generated from the runs you have planned.
2. If more capacity is required:
   a. Empty the waste carboy where the two green system waste lines are directed.
   b. Empty the vacuum waste flask.

c Check that the two green waste lines are not dipping in waste fluid, but merely sticking out of the carboy lid.

Starting the system

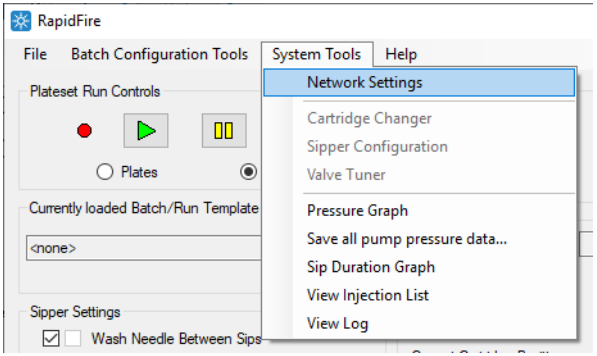
To start the system:

1. Press the two green power buttons on the back of the frame to turn on the system power.
2. Turn on the quaternary pumps, peristaltic pump, temperature control unit (if present), computer, and vacuum source.
3. Double-click the **RapidFire Control Panel** desktop icon.
4. Click **File > Choose CFGs**.
5. Select the configuration folder under **C:\Agilent\RapidFire**.
6. Click **Start** in the RapidFire Control Panel.

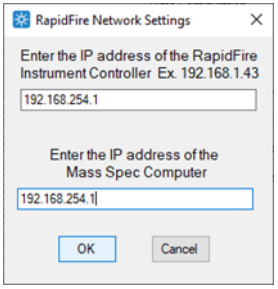


7. Double-click the **RapidFire UI** desktop icon.

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



9 Verify that the IP addresses for the RapidFire computer and the mass spectrometer computer are correct:



To find the computer IP address:

a Click the **Start** icon in the Windows desktop, type **Run**, and select the **Command Prompt App**.

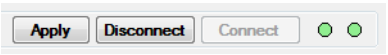
b In the **Command Prompt** window, type **ipconfig-all** and press **Enter**.

NOTE

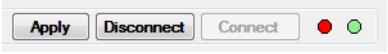
If you are controlling the mass spectrometer and RapidFire on one computer, their IP addresses will be the same.

10 Click **Connect** in the main window to connect to the program server.

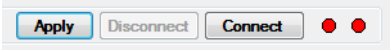
The indicator lights next to Connect turn green.



If one indicator is red, the system is connected but the door is open or the emergency stop button is pressed.



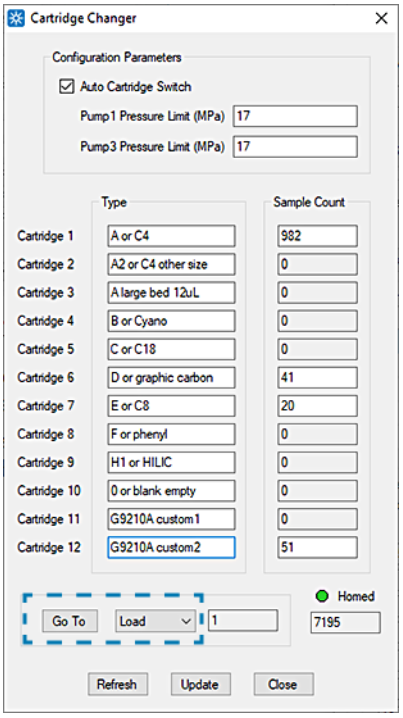
If both lights are red, the system is not connected.



Loading and homing the cartridge changer

To load and home the cartridge changer:

1. Click **System Tools > Cartridge Changer** to open the Cartridge Changer dialog box.
2. Select **Load** and click **Go To** to move the changer to the load position.



3 Load up to 12 new cartridges in the holder:

NOTE

Always put one cartridge in slot 1 as the cartridge changer travels to this position after the homing routine is complete.

a Remove the protective packaging and insert the new cartridges in the slots, with the notch end facing up.

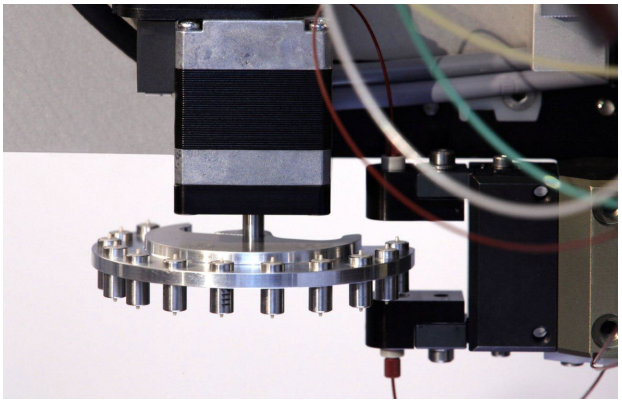


Figure 1. Cartridge changer with 12 cartridges

b Update the information in the Cartridge Changer dialog box.

c 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.

d Click **Update**.

e In the Cartridge Changer dialog box, select **Home** from the **Go To** list and click **Go To** to home the changer.

The Homed indicator turns green.

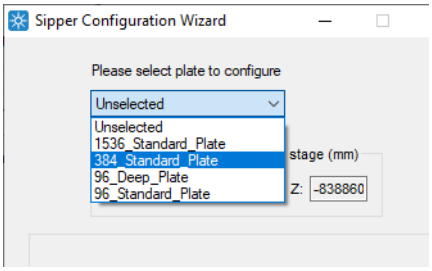
Homing the stage

After you set the stage teachpoints for your particular plate type, you only need to home the stage when performing a run.

To home the stage:

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

2 Make sure the plate type you are using is selected in the **plate to configure** field.



3 Click **Home** and wait for all three axis stages (*x*, *y*, and *z*) to get to home position (0,0,0).

4 Do one of the following:

- If you have not yet set the stage teachpoints, go to **"Setting the stage teachpoints"** on page 18.
- If you have set the stage teachpoints, close the wizard and go to **"Checking the nanovalves"** on page 45 to continue with preparing for a run.

Setting the stage teachpoints

About setting the stage teachpoints

You teach the RapidFire system stage positions when you do the following:

- Use a new plate type
- Replace the sipper tube or guiding needle

Setting the stage teachpoints for a plate requires that the plate is first predefined in the software. The RapidFire software has four predefined labware types (.platecfg files) that can be used to perform the teaching:

- 96-well standard microplate
- 96-deep well microplate
- 384-well standard microplate
- 1536-well standard microplate

NOTE

Using 1536-well microplates requires smaller diameter injection loop and sipper tube configuration to minimize sample volume. See **"Minimizing the required sample volume"** on page 62 for more information.

About the Sipper Configuration Wizard

The software uses the data from the first four steps of the wizard (Touchpoint 1, Touchpoint 2, Touchpoint 3, and Touchpoint Z) to calculate all of the stage teachpoints including the plate teachpoints, wash station teachpoints, and matrix teachpoints.

Once you click **Set** for the calculated teachpoints, you can verify and adjust the calculated teachpoints by continuing with the wizard, or you can skip the verification by clicking **Next**, without clicking **Set**, in the relevant dialog box.

You also have the option of setting all the teachpoints manually by skipping the first four steps of the wizard. To skip a step, click **Next** in the dialog box.

Before you begin

Make sure you have the following:

- An empty plate of the type you are teaching and, if applicable, four empty matrix bottles
- Stage teaching jig and attachment wire with clip

Use the following workflow to set the stage teachpoints.

Step	Task	See
1	Set up the teaching jig and teach the four touchpoints.	**"Setting up the system for teaching and teaching the four touchpoints"** on page 20
2	View and accept or edit the calculated teachpoints.	**"Accepting or editing the calculated teachpoints"** on page 30
3	Verify the wash station, corner well, and matrix position teachpoints.	**"Verifying the calculated teachpoints"** on page 34

Setting up the system for teaching and teaching the four touchpoints

In this first part of the wizard, you will do the following:

Step	Task	See
1	Open the wizard and install the teaching jig.	**"Opening the Sipper Configuration Wizard and installing the teaching jig"** on page 21
2	Set the Sipper Safe Height and teach the four touchpoints.	**"Setting the Sipper Safe Height and teaching the four touchpoints"** on page 24
3	Prepare to verify the calculated teachpoints.	**"Preparing to verify the calculated teachpoints"** on page 30

Opening the Sipper Configuration Wizard and installing the teaching jig

To open the wizard and install the jig:

1 Open the Sipper Configuration Wizard (see **"Homing the stage"** on page 18) and home the stage.

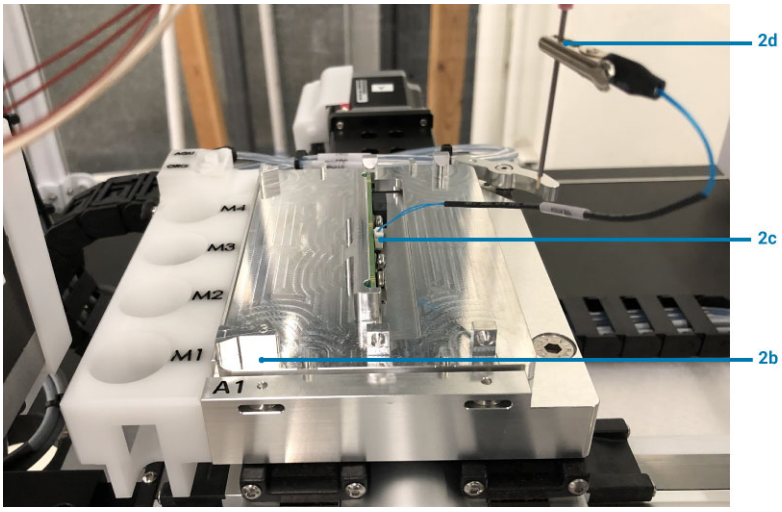
2 Install the stage teaching jig:

a Open the doors to disengage the stage motors.

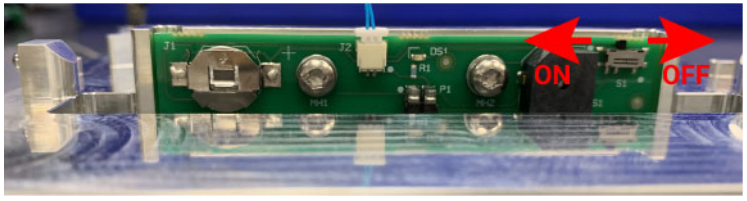
b Place the teaching jig on the stage and make sure teachpoints labeled 1 and 3 on the jig are near the A1 corner of the stage.

c Connect one end of the jig wire with the connector pin to the jig itself.

d With the alligator clip, attach the other end of the jig wire to the sipper assembly near the top of the needle.

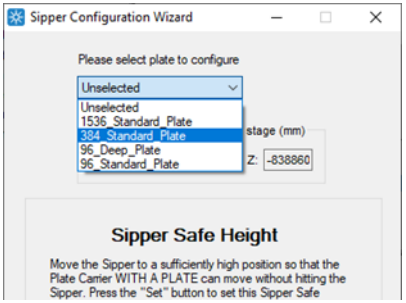


e Turn on the power to the jig.



3 If you are using matrix bottles, place them in the four holes marked M.

4 Select the plate type from the **plate to configure** list to activate the wizard.



Setting the Sipper Safe Height and teaching the four touchpoints

CAUTION

Set the Sipper Safe Height before starting operation to avoid damage to the equipment.

To set the Sipper Safe Height and teach the four touchpoints:

1 Set the Sipper Safe Height as follows:

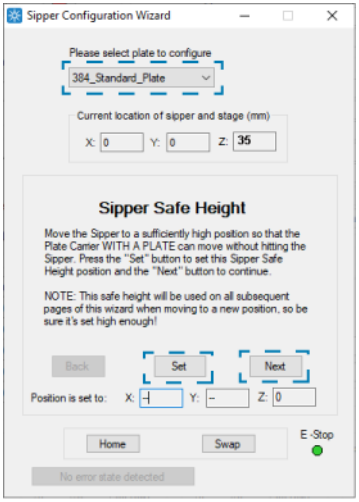
a Turn the black knob on the end of the *z*-stage to adjust the *z*-axis so that the bottom of the sipper clears all potential obstacles (such as the plate, wash station, and matrix bottles) by about 0.5 cm (1/4 inch).

NOTE

Perform this step regardless of where the stage moves along the *x*- or *y*-axis.

b Close the door to engage the stage motors.

c Click **Set** and then **Next** in the Sipper Configuration Wizard.



2 Configure **Touchpoint 1 Position**:

a Open the doors and manually adjust the position of the stage in the *x*-, *y*- and *z*-axes so that the sipper is adjacent to the Touchpoint 1 mark on the jig, as the following figure shows.

b Continue to adjust the *x*-axis (side-to-side) of the stage until the sipper tip contacts the teaching jig and causes the light to flicker and buzzer to sound intermittently on the jig.

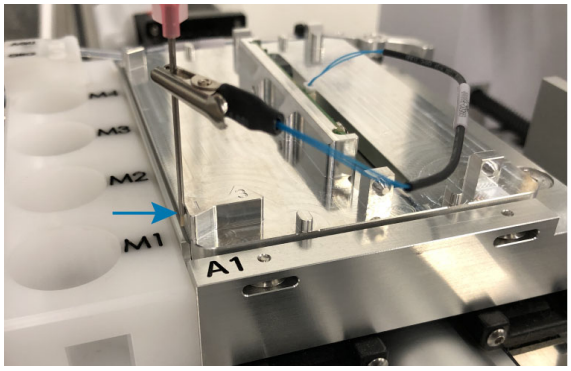
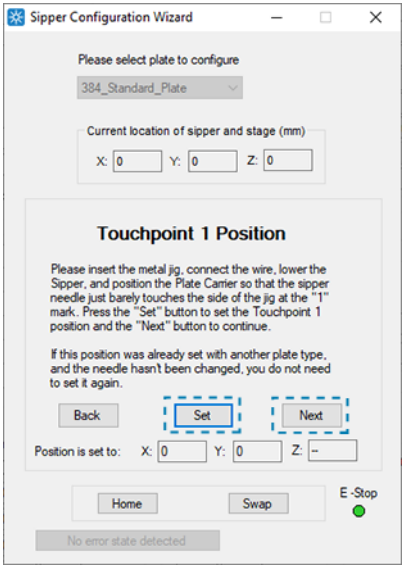


Figure 2. Location of Touchpoint 1 position

c Close the door to engage the stage motors.

d Click **Set** and then **Next** in the Sipper Configuration Wizard.



3 Configure **Touchpoint 2 Position**:

a Open the doors to disengage the motors.

b Manually adjust the *x*-, *y*-, and *z*-axes of the stage so that the sipper is adjacent to the Touchpoint 2 mark on the jig, as the following figure shows.

c Continue to adjust the *x*-axis of the stage until the sipper tip contacts the teaching jig and causes the light to flicker and buzzer to sound intermittently on the jig.

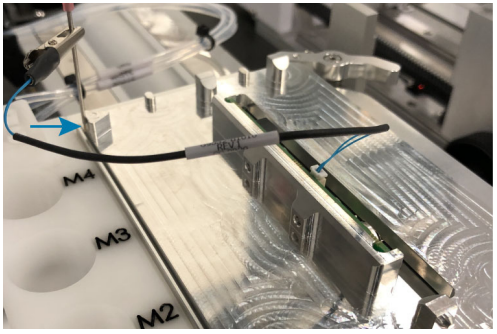
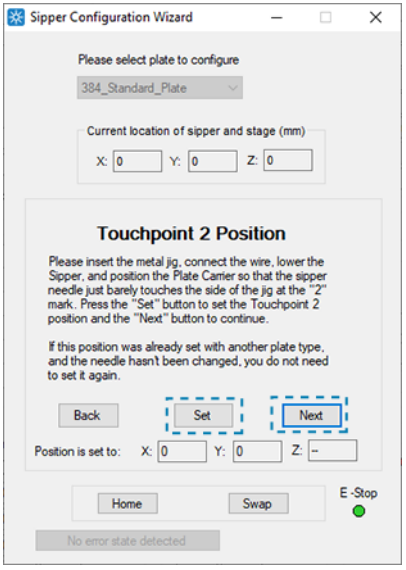


Figure 3. Location of Touchpoint 2 position

d Close the door to engage the stage motors.

e Click **Set** and then **Next** in the Sipper Configuration Wizard.



4 Configure **Touchpoint 3 Position**:

a Open the doors to disengage the motors.

b Manually adjust the *x*-, *y*-, and *z*-axes of the stage so that the sipper is adjacent to the Touchpoint 3 mark on the jig, as the following figure shows.

c Continue to adjust the *y*-axis backward and forward of the stage until the sipper tip contacts the teaching jig and causes the light to flicker and buzzer to sound intermittently on the jig.

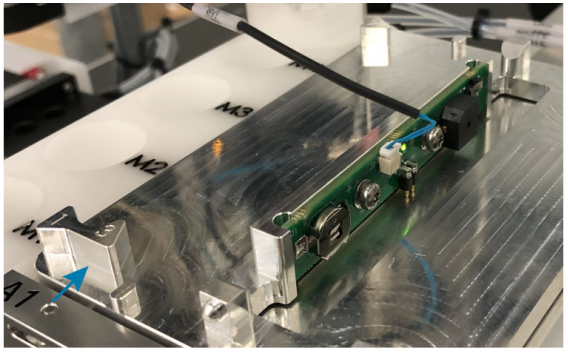
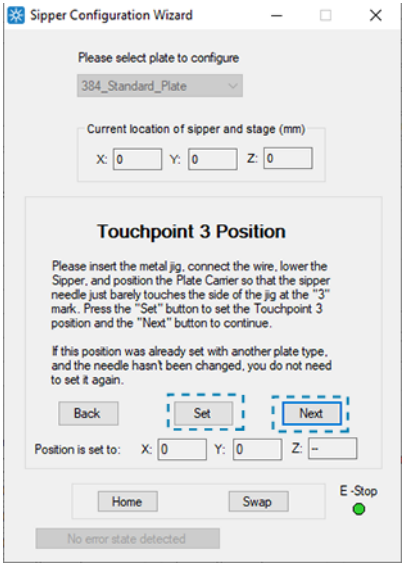


Figure 4. Location of Touchpoint 3 position

d Close the door to engage the stage motors.

e Click **Set** and **Next** in the Sipper Configuration Wizard.



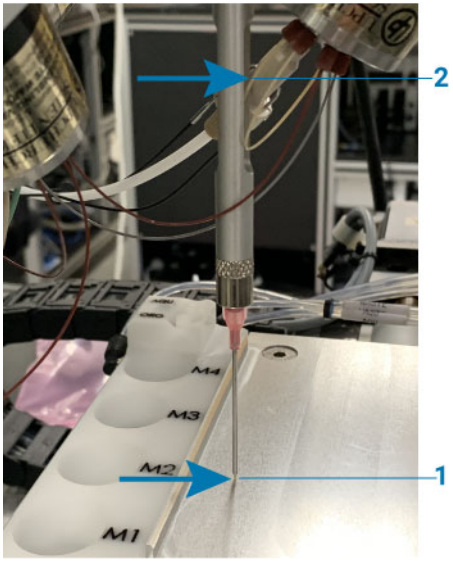
5 Configure the **Touchpoint Z Position**:

a Open the doors to disengage the motors.

b Remove the jig from the stage.

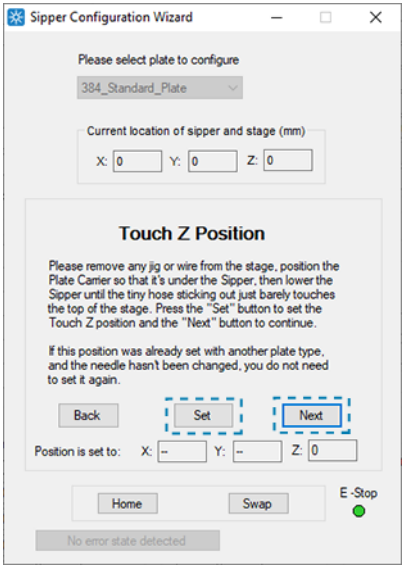
c Manually adjust the *x*-, *y*-, and *z*-axes so that the sipper is above the empty stage.

d Slowly lower the sipper in the *z*-axis (vertical) until the tubing extending over the tip of the sipper needle touches the surface of the stage (1) and the tubing flexes (2).



e Close the doors to engage the motors.

f Click **Set** and **Next** in the Sipper Configuration Wizard.



Preparing to verify the calculated teachpoints

To prepare to verify the calculated teachpoints:

Open the doors and install an empty sample plate on the stage. Ensure the plate is securely positioned, and then close the doors and proceed to **"Accepting or editing the calculated teachpoints"** on page 30.

Accepting or editing the calculated teachpoints

In this part of the Wizard, the calculated teachpoints for the corner wells, wash stations, and matrix bottles are displayed and you can accept the values or edit them. You will do the following:

Step	Task	See...
1	View the calculated teachpoints for the corner wells and accept or edit them.	**"Viewing the calculated teachpoints for the corner wells"** on page 31
2	View the calculated teachpoints for the wash station teachpoints and accept or edit them.	**"Viewing the calculated wash station teachpoints"** on page 32
3	View the calculated matrix teachpoints and accept or edit them.	**"Viewing the calculated matrix teachponts"** on page 33

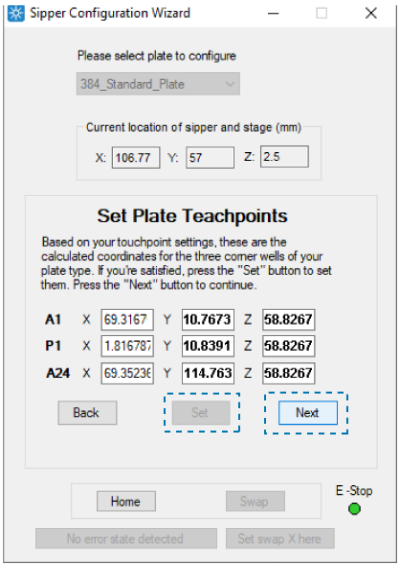
Viewing the calculated teachpoints for the corner wells

To accept or edit the corner well teachpoints:

1 In the **Set Plate Teachpoints** area, view the calculated teachpoints for three corner wells of the plate.

The teachpoints are calculated for the following corner wells, depending on the plate type you are teaching:

Plate size	First corner well	Second corner well	Third corner well
96-well	A1	H1	A12
384-well	A1	P1	A12
1536-well	A1	FF1	A48



2 Choose one of the following options:

- Accept these values and click **Set** and **Next** to continue to the calculated Wash Station teachpoints step of the wizard.
- Edit one or more of the values and when finished, close the doors, and click **Set** and then **Next** to continue to the calculated Wash Station teachpoints step of the wizard.

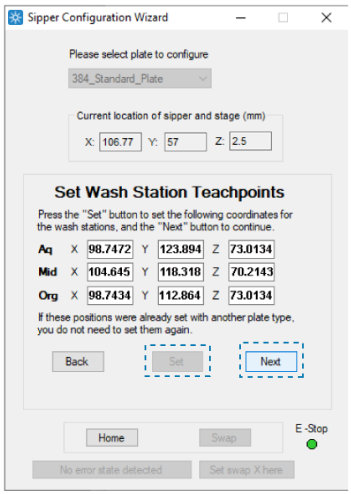
NOTE

In the following steps, the wizard will go to each of these teachpoints and you can verify or edit each teachpoint.

Viewing the calculated wash station teachpoints

To accept or edit the wash station teachpoints:

1 In the **Set Wash Station Teachpoints** area, view the teachpoints for the Aqueous, Middle, and Organic wash stations.



2 Choose one of the following options:

- Accept these values and click **Set** and **Next** to continue to the calculated matrix position teachpoints of the wizard.
- Edit one or more of the values and when finished, close the doors, and click **Set** and then **Next** to continue to the calculated matrix teachpoints of the wizard.

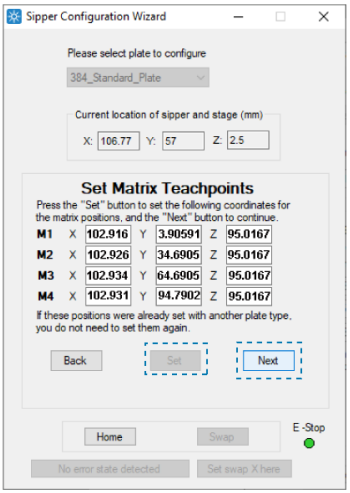
NOTE

In the following steps, the wizard will go to each of these teachpoints and you can verify or edit each teachpoint.

Viewing the calculated matrix teachponts

To view or edit the matrix teachpoints:

1 In the **Set Matrix Teachpoints** area, view the calculated teachpoints for the matrix bottle positions.



2 Choose one of the following options:

- Accept these values and click **Set** and **Next** to move the stage to the calculated *x*- and *y*-coordinates of the **Aqueous Wash Station** teachpoint.
- Edit one or more of the values and when finished, close the doors, and click **Set** and then **Next** to move the stage to the **Aqueous Wash Station** teachpoint.

Verifying the calculated teachpoints

In this part of the wizard you verify or edit the calculated teachpoints. If you do not want to change a teachpoint, click **Next** to advance to the next step without changing the teachpoint. You will do the following:

Step	Task	See
1	Verify the wash station teachpoints.	**"Verifying the wash station teachpoints"** on page 35
2	Verify the corner well teachpoints.	**"Verifying the corner well teachpoints"** on page 38
3	Verify the matrix position teachpoints.	**"Verifying the matrix position teachpoints"** on page 41

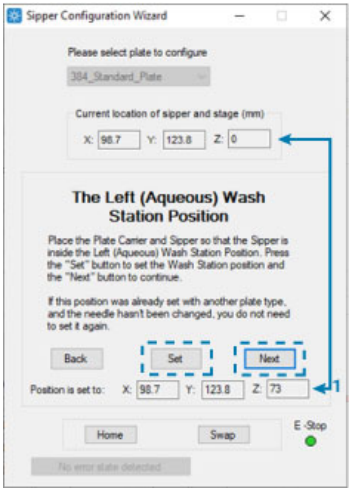
Verifying the wash station teachpoints

To verify the wash station teachpoints:

1 Verify the **Aqueous Wash Station** teachpoint as follows:

- **a** Ensure the sipper is situated over the center of the Aqueous Wash Station chimney in the *x*- and *y*-axes.
- **b** To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.
- **c** Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Wash Station Teachpoint area in **"Viewing the calculated wash station teachpoints"** on page 32).
- **d** Adjust the *z*-axis depth, if required.

  The sipper tube should reach a depth sufficient to wash the outside of the sipper that is exposed to well samples during a run. The depth required depends on the well size and the sample volume.
- **e** When the position is correct, close the doors, and click **Set** and **Next** to move the stage to the calculated *x*- and *y*-coordinates of the **Organic Wash Station** chimney teachpoint.



2 Verify the **Organic Wash Station** teachpoint as follows:

a Ensure the sipper is situated over the center of the Organic Wash Station chimney in the *x*- and *y*-axes.

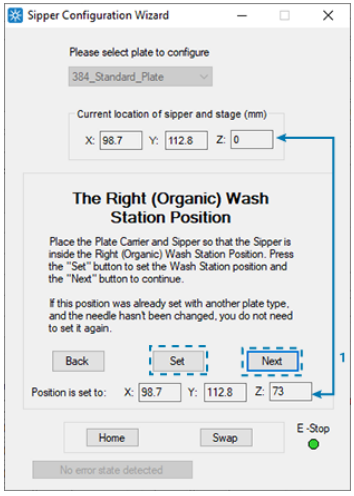
b To adjust, open the doors and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Wash Station Teachpoint area in **"Viewing the calculated wash station teachpoints"** on page 32).

d Adjust the *z-axis* depth, if required.

The sipper tube should reach a depth sufficient to wash the outside of the sipper that is exposed to well samples during a run. The depth required depends on the well size and the sample volume.

e When the position is correct, close the doors and click **Set** and **Next** to move the stage to the calculated *x*- and *y*- coordinates of the **Middle Wash Station** chimney teachpoint.



3 Verify the **Middle Wash Station** teachpoint as follows:

a Ensure the sipper is situated over the center of the Middle Wash Station chimney in the *x*- and *y*-axes.

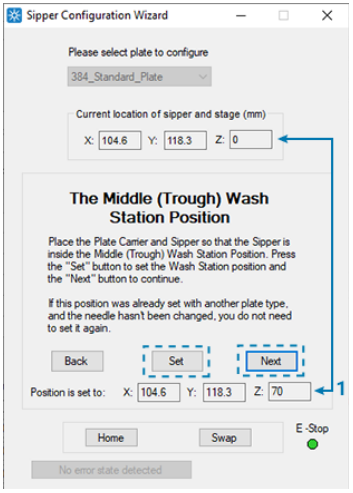
b To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Wash Station Teachpoints area in **"Viewing the calculated wash station teachpoints"** on page 32).

d Adjust the *z*-axis depth, if required.

The sipper tube should enter the middle wash station to a depth past the inner hole so that when the sipper purges, fluid will not escape.

e When the position is correct, close the doors, and click **Set** and **Next** to move the stage to the calculated *x*- and *y*-coordinates of the corner well teachpoint, H1 for a 96-well, P1 for a 384-well, and FF1 for a 1536-well plate.



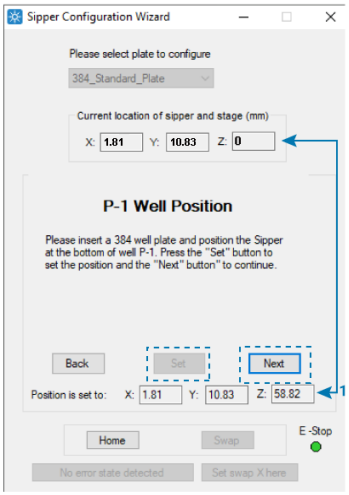
Verifying the corner well teachpoints

To verify the corner well teachpoints:

1 Verify the sipper teachpoint for the first corner well as follows:

- **a** Ensure the sipper is situated over the center of the well in the *x*- and *y*-axes.
- **b** To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.
- **c** Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Plate Teachpoints area in **"Viewing the calculated teachpoints for the corner wells"** on page 31).
- **d** Adjust the *z*-axis depth, if required, and then close the doors.

  The sipper tube should be fully immersed when sampling occurs.
- **e** Click **Set** and **Next** to move the stage to the calculated *x*- and *y*-coordinates of the corner well teachpoint, A1.



2 Verify the **A1 well Position** teachpoint:

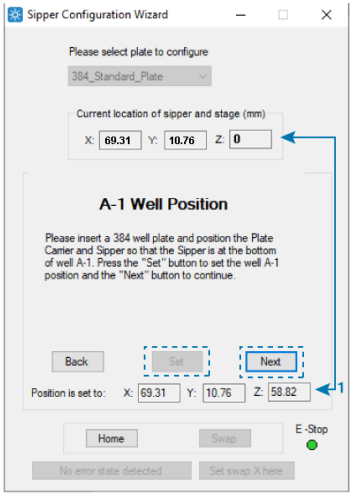
a Ensure the sipper is situated over the center of the well in the *x*- and *y*-axes.

b To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Plate Teachpoints area in **"Viewing the calculated teachpoints for the corner wells"** on page 31).

d Adjust the *z*-axis depth, if required, and then close the doors. The sipper tube should be fully immersed when sampling occurs.

e Click **Set** and **Next** to move the stage to the calculated *x*- and *y*-coordinates of the third corner well teachpoint, A12, A24, or A48.



3 Verify the third corner well teachpoint:

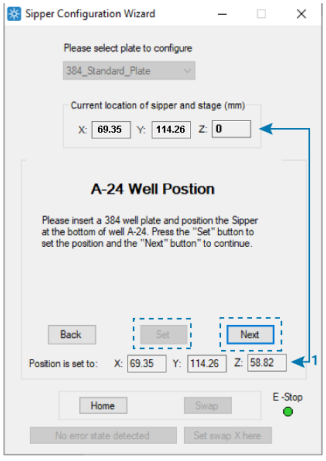
a Ensure the sipper is situated over the center of the well in the *x*- and *y*-axes.

b To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Plate Teachpoints area in **"Viewing the calculated teachpoints for the corner wells"** on page 31).

d Adjust the *z*-axis depth, if required, and then close the doors. The sipper tube should be fully immersed when sampling occurs.

e Click **Set** and **Next** to move the stage to the calculated *x*- and *y*-coordinates of the **Matrix 1 Position** teachpoint.



Verifying the matrix position teachpoints

To verify the matrix position teachpoints:

1 Verify the **Matrix 1 Position** teachpoint:

a Ensure the sipper is situated over the center of the bottle in the *x*- and *y*-axes.

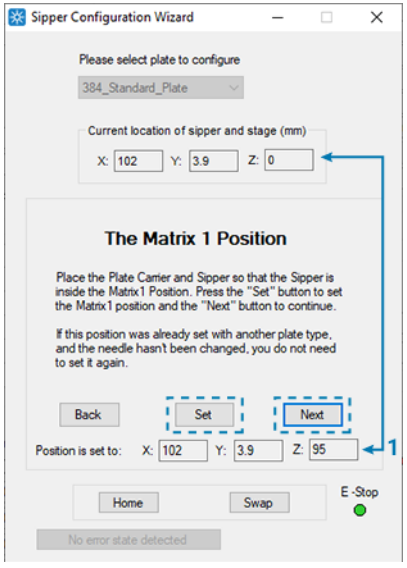
b To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Matrix Teachpoints area in **"Viewing the calculated matrix teachponts"** on page 33).

a Adjust the *z*-axis depth, if required, and then close the doors.

The sipper tube depth should be adequate to reach the maximum amount of sample.

b Click **Next** to move the stage to the calculated *x*- and *y*-coordinates of the **Matrix Position 2** teachpoint.



2 Verify the **Matrix 2 Position** teachpoint:

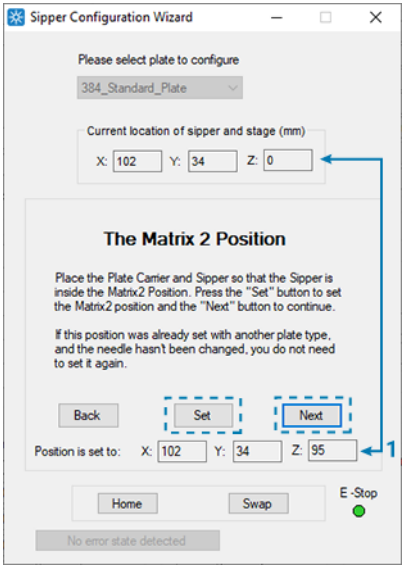
a Ensure the sipper is situated over the center of the bottle in the *x*- and *y*-axes.

b To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Matrix Teachpoints area in **"Viewing the calculated matrix teachponts"** on page 33).

d Adjust the *z*-axis depth, if required, and then close the doors. The sipper tube depth should be adequate to reach the maximum amount of sample.

e Click **Next** to move the stage to the calculated *x*- and *y*-coordinates of the **Matrix Position 3** teachpoint.



3 Verify the **Matrix 3 Position** teachpoint:

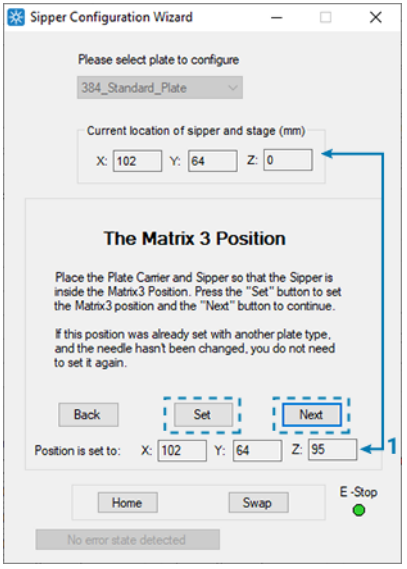
a Ensure the sipper is situated over the center of the bottle in the *x*- and *y*-axes.

b To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Matrix Teachpoints area in **"Viewing the calculated matrix teachponts"** on page 33).

d Adjust the *z*-axis depth, if required, and then close the doors. The sipper tube depth should be adequate to reach the maximum amount of sample.

e Click **Next** to move the stage to the calculated *x*- and *y*-coordinates of the **Matrix Position 4** teachpoint.



4 Verify the **Matrix 4 Position** teachpoint:

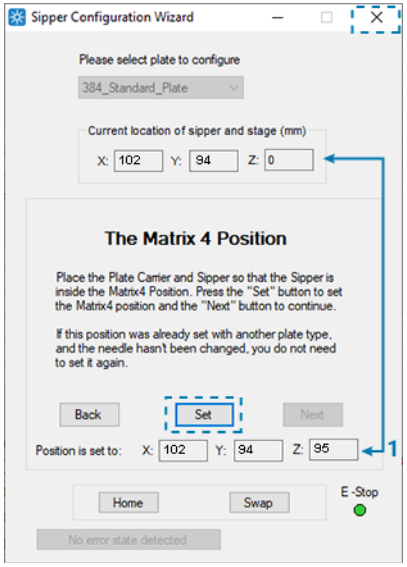
a Ensure the sipper is situated over the center of the bottle in the *x*- and *y*-axes.

b To adjust, open the doors, and manually move the stage in the *x*- and *y*-axes to the correct location.

c Manually move the stage in the *z*-axis from its *z*-safe height to the calculated set value as shown below (1) (also shown in the Set Matrix Teachpoints area in **"Verifying the matrix position teachpoints"** on page 41).

d Adjust the *z*-depth, if required, and then close the doors. The sipper tube depth should be adequate to reach the maximum amount of sample.

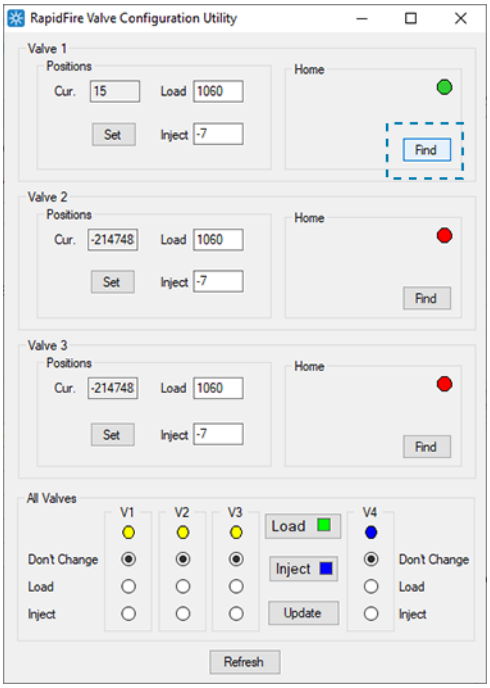
e Click **Set** and then close the Sipper Configuration Wizard.



Checking the nanovalves

To check the nanovalves:

1 Click **System Tools > Valve Tuner** to open the **Valve Configuration Utility** dialog box.



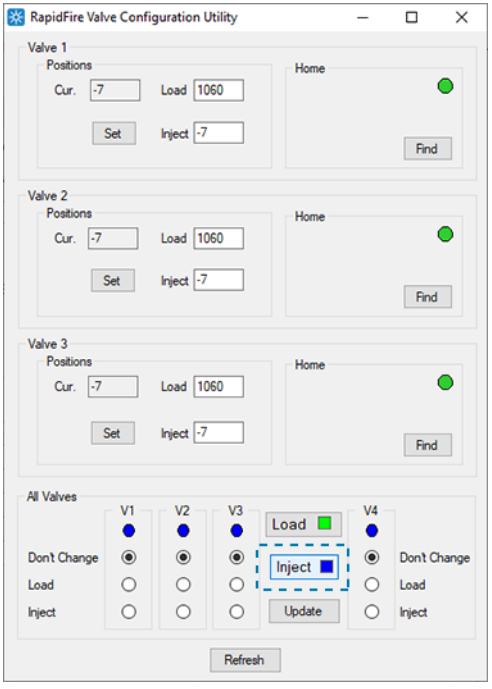
2 Click **Find** next to each valve to home it. The Home status light turns green when the valve is homed.

3 Click **Inject** and **Load** to change the status of the valves and to verify that the change in status is smooth and quiet.

The valve status lights are green when they are in the Load position and the values in the Cur field of the Positions area should be close to zero.

The valve status lights are blue when they are in the Inject position and the values in the Cur field of the Positions area should be high >1000.

If the status lights for a valve (V1, V2, V3) are yellow and the Home status light is red, click **Find** to home the valve.



Preparing the peristaltic pump

About the peristaltic pump

Your RapidFire 400 System has one of two types of peristaltic pumps:

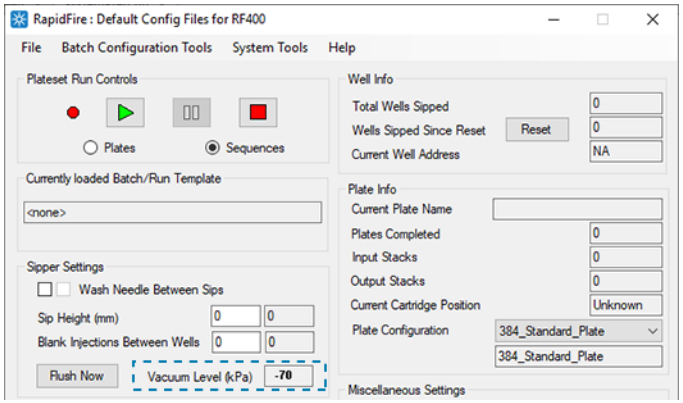
- ISM peristaltic pump whose flow rate is controlled by buttons on the device.

- L-100 peristaltic pump whose flow rate is controlled in the RapidFire software.

To prepare the peristaltic pump:

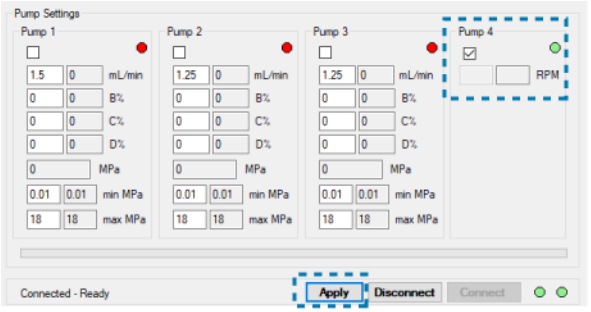
1 Ensure the pump is turned on.

2 Ensure the vacuum is on and at least -70 kPa.

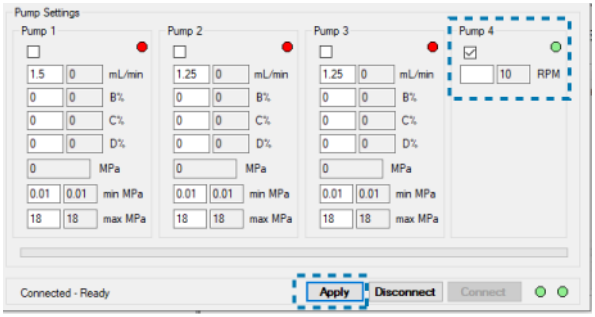


3 In the RapidFire main software window, select the **Pump 4** check box and click **Apply** to start the flow.

For ISM peristaltic pumps, the flow rate control is unavailable in the software.



For the L-100 peristaltic pumps, the flow rate sets to the default 10 RPM.



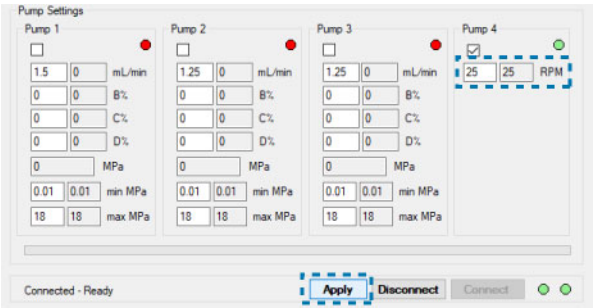
4 Verify the pump is running in the clockwise direction.

5 Set the pump flow rate:

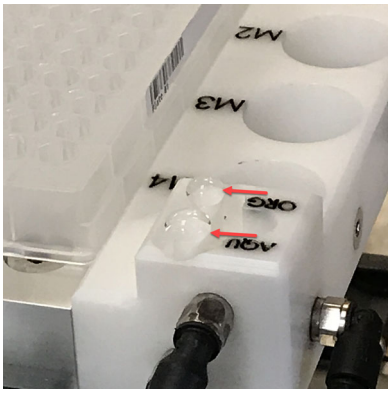
a Ensure that fluid is moving through the tubing lines that connect Pump 4 to the wash station.

b For ISM pumps, adjust the pump flow by pressing the up/down arrow buttons on the pump control panel.

c For L-100 pumps, adjust the pump flow by entering a number in the **RPM** field and clicking **Apply** in the RapidFire main window software.



d When the fluid starts to flow into the wash station, adjust the flow rate until droplets of liquid adhere to the aqueous and organic wash inlet tubes for about 6-8 seconds.



6 Ensure that fluid is being aspirated from the center trough sufficiently and no overflow occurs on the wash station.

Preparing the quaternary pumps

This procedure primes the pumps and removes air bubbles in the fluidic lines.

To prepare the quaternary pumps:

1. Turn the diversionary valve (black knob) counterclockwise 90-180 degrees to open the valve.
2. In the RapidFire software window, select the check boxes for Pumps 1, 2, and 3, and then click **Apply**.
3. Verify that the composition for channel B, C, and D is 0%.
4. Set the flow rate for each pump to 5 mL/min, click **Apply**, and let the pumps operate for 2 minutes to prime channel A.
5. Change the solvent composition by entering 100% for B and 0% for A, C, and D, click **Apply**, and allow the pumps to operate for 2 minutes to prime channel B.
6. Change the solvent composition by entering 100% for C and 0% for A, B, and D, click **Apply**, and allow the pumps to operate for 2 minutes to prime channel C.
7. Change the solvent composition by entering 100% for D and 0% for A, B, and C click **Apply**, and allow the pumps to operate for 2 minutes to prime channel D.
8. Set the composition and flow rates to your assay-specific values, for example 100% A at 1.25 mL/min, and click **Apply**.
9. Turn the black knob clockwise until it is finger-tight to close the diversionary valve.
10. Check the pressure readings:
    a. Click **System Tools > Valve Tuner** to open the Valve Configuration Utility dialog box.
    b. Select the **Inject** position for valves 1, 2, and 3 and click **System Tools > Pressure Graph**, to check the pressure.
    c. Select the **Load** position for valves 1,2, and 3 and click **System Tools > Pressure Graph**, to check the pressure.

    The maximum pressure is 20 MPa. The following table lists typical pressure values.

Table 4. Typical pressure values

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

If the value is outside the typical range shown for each pump at the load or inject position:

- Check the flow path for leaks (low pressure) or clogs (high pressure), for example, a plugged cartridge.
- Verify you are using proper and miscible solvents.

Preparing the sipper

This procedure uses pump 1 to flush the contents of the sipper and its tubing into the wash station drain.

To prepare the sipper:

In the RapidFire software window, click **Flush Now** in the **Sipper Settings** area.

The following occurs:

a Pump 1 turns on.

b The stage moves so that the sipper is positioned over the wash station drain.

c Valve 4 actuates to the Load position.

d The flow rate for pump 1 increases to 8 mL/min and maintains for one minute after which it returns to its previous setting.

e Pump 1 turns off.

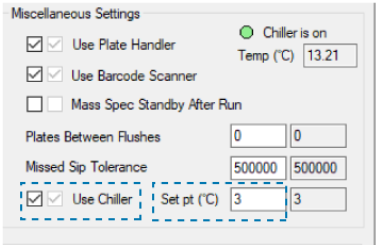
f Valve 4 actuates to the Inject position.

g The stage moves to the Swap position.

Preparing the temperature control unit

To turn on the temperature control unit:

1. Select the **Use Chiller** check box if you want to maintain a temperature between 5-25 °C during the run.
2. In the **Set pt(°C)** field, enter the temperature that you want the unit to maintain during the run.
3. Click **Apply** to turn on the temperature control unit.



Preparing the TOF or Q-TOF mass spectrometer

Before you start

If you are using one computer to control both the RapidFire instrument and the mass spectrometer:

- Ensure Mass Hunter Data Acquisition software 10.00.xx or later is installed on the computer.

If you are using two computers, one to control the RapidFire instrument and one to control the mass spectrometer:

- Ensure Mass Hunter Data Acquisition software 10.00.xx or later is installed on the computer controlling the mass spectrometer.
- Ensure RapidFire Communicator software version 6.0 or later is installed on the computer controlling the mass spectrometer.

To prepare the TOF or Q-TOF mass spectrometer:

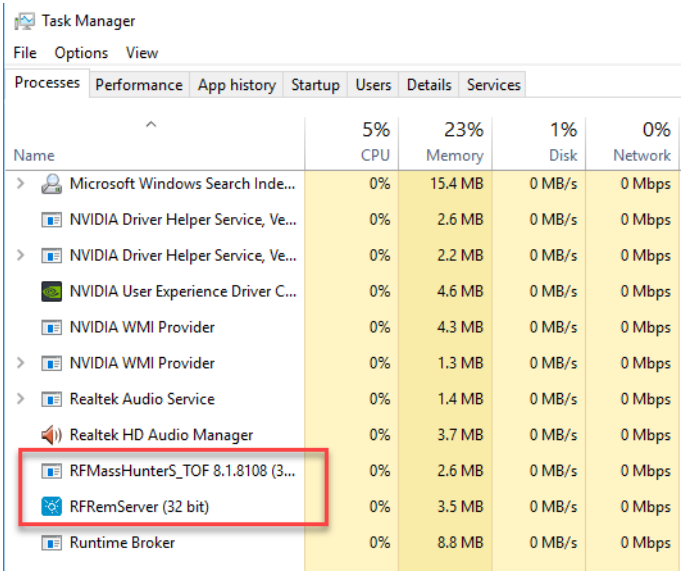
1 If you are using one computer to control the RapidFire instrument and the mass spectrometer, go to **step 2**.

If you are using two computers to control the RapidFire instrument and the mass spectrometer:

a Open RapidFire Communicator by double-clicking the desktop icon ( ).

b Open the Windows Task Manager and verify the following processes are running:

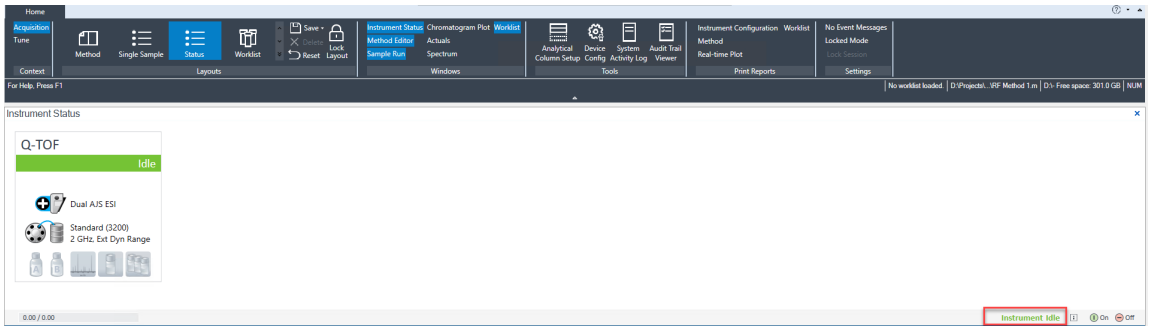
- RFRemServer.exe
- RFMasshunterS_TOF.exe



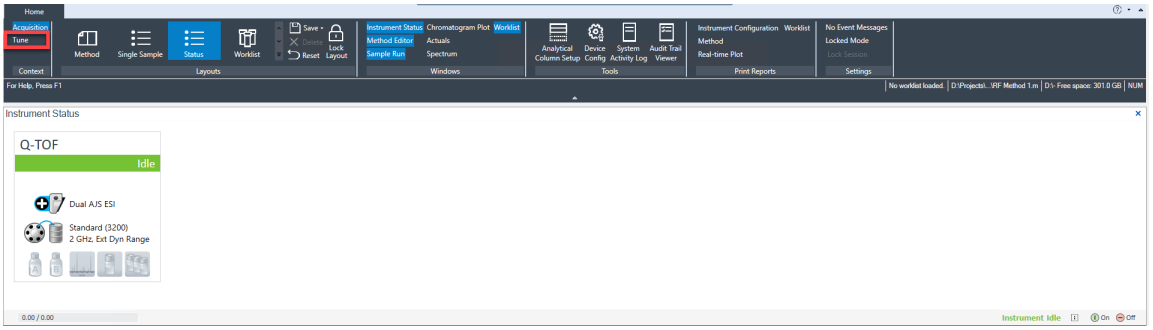
2 Start the Mass Hunter Data Acquisition software by double-clicking the Data Acquisition desktop icon.

3 To turn on the detector, click **On** in the Q-TOF device pane or in the status line for the Instrument Status area.

The status indicator of the device in the **Status** area turns from teal (Standby, connected), to yellow (Not Ready, preparing), and then to green (Ready, idle).



4 Select **Tune** from the **Context** area.



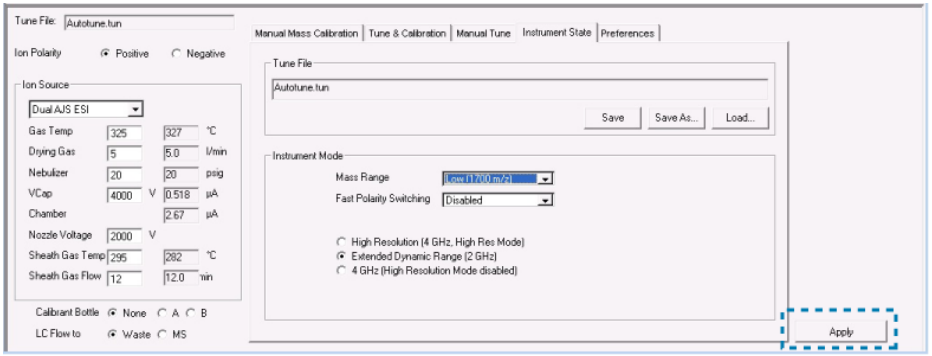
5 Set the mass and resolution parameters for your analysis in the Instrument State tab in the Tune window.

Note that:

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

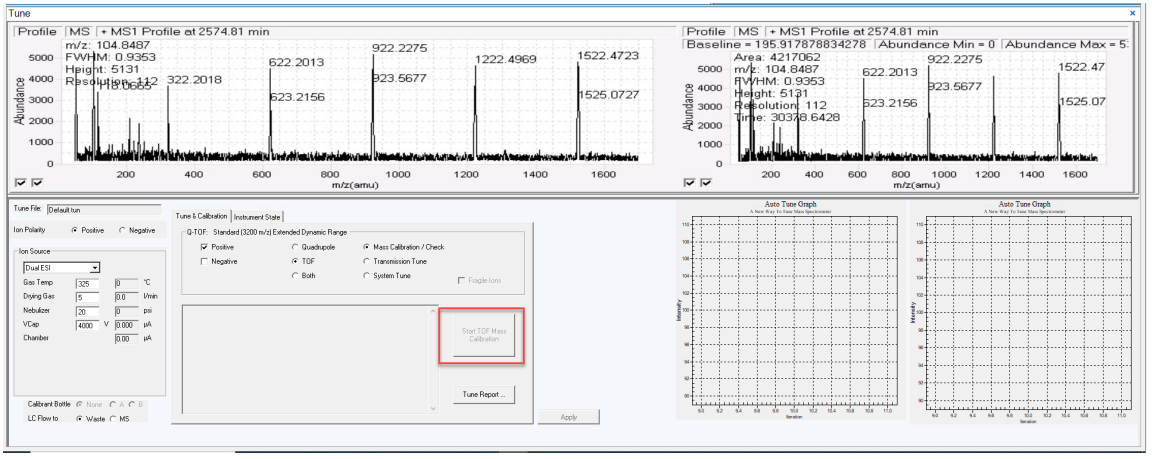
a Click **Apply** on the bottom right.

b Wait for the system to equilibrate.

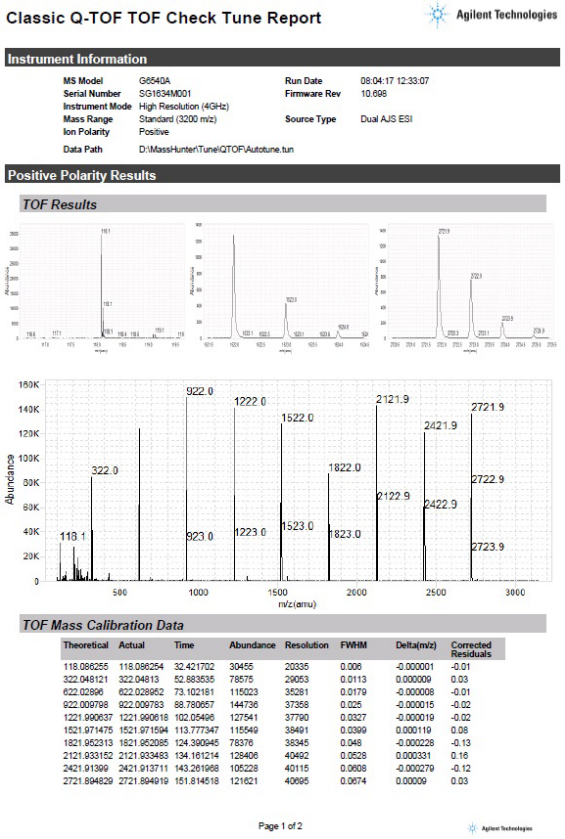


6 Calibrate the mass spectrometer:

- **a** Set the location of the **Calibrant Bottle**, for example B, and wait for the mass-over-charge spectrum to stabilize.
- **b** Click the **Tune & Calibration** tab.
- **c** Select the **Polarity** to use. You can select both, if desired.
- **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 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	38491	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	128406	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 to **None**.

7 Select **Acquisition** from the **Context** list.

If prompted, confirm that you want to operate under the modified Instrument State.

NOTE

DO NOT modify the Tune File.

8 Click **Method > Open** to open a method (file type is **.m**) or set the 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 TOF or Q-TOF tabs. The following figures show example screens.

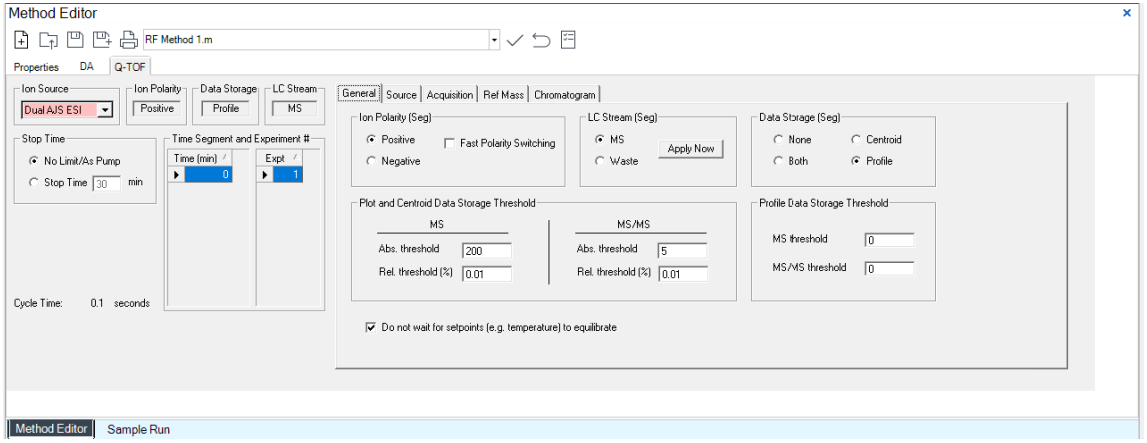


Figure 5. General tab example

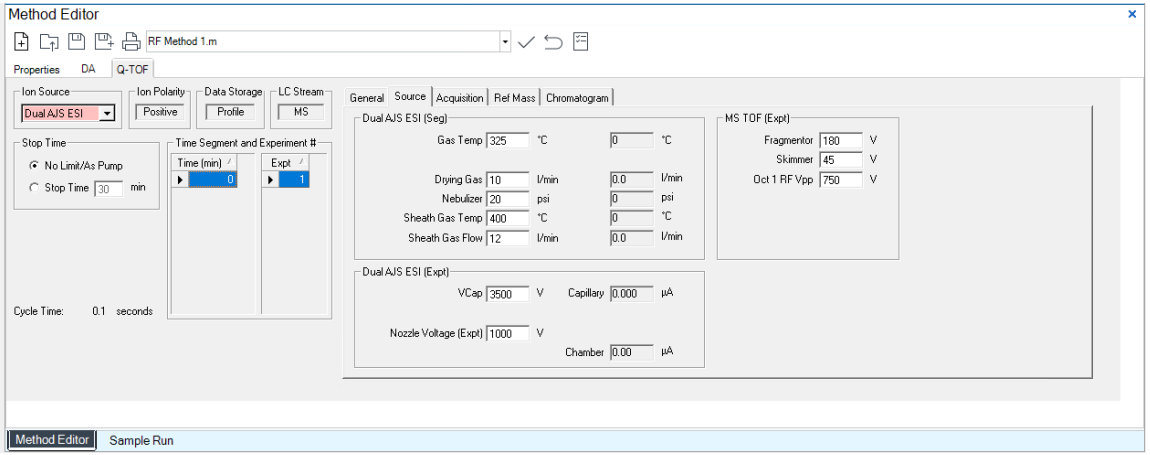


Figure 6. Source tab example

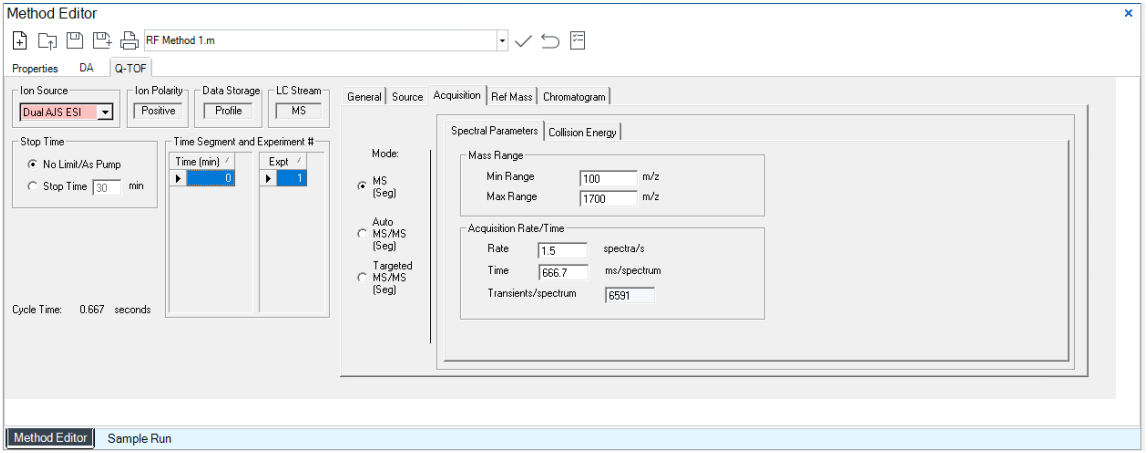


Figure 7. Acquisition tab example

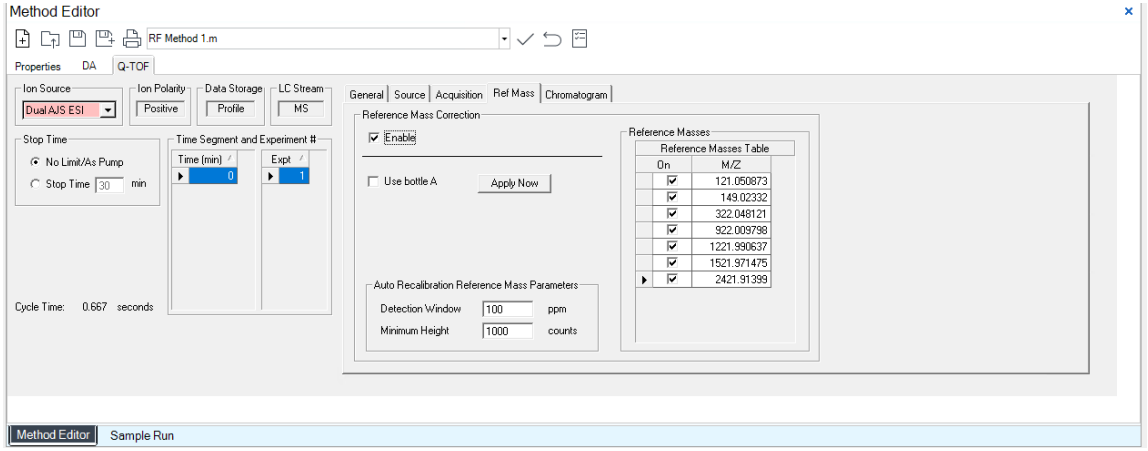


Figure 8. Reference Mass example

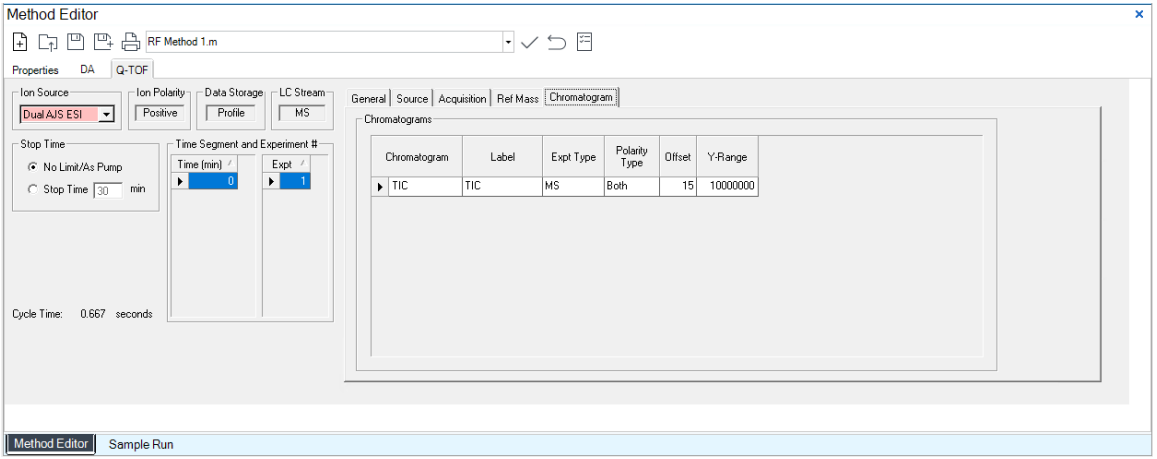
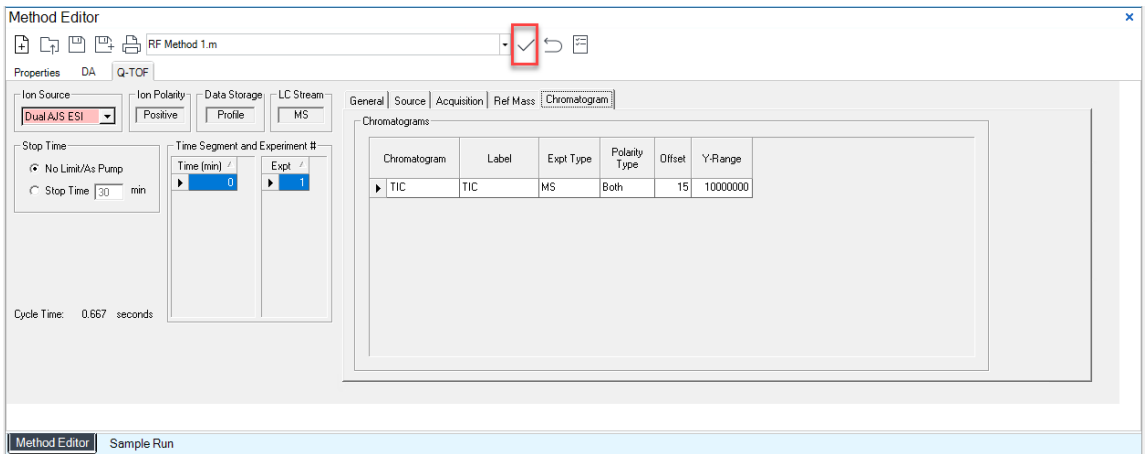


Figure 9. Chromatogram tab example

10 When you have finished setting the parameters, click the check mark (✓) in the toolbar area of the Method Editor window.



11 Save the method.

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

Preparing the TQ or Ultivo TQ mass spectrometers

Before you start

If you are using one computer to control both the RapidFire instrument and the mass spectrometer:

- Ensure Mass Hunter Data Acquisition software 10.00.xx or later is installed on the computer.

If you are using two computers, one to control the RapidFire instrument and one to control the mass spectrometer:

- Ensure Mass Hunter Data Acquisition software 10.00.xx or later is installed on the computer controlling the mass spectrometer.
- Ensure RapidFire Communicator software version 6.0 or later is installed on the computer controlling the mass spectrometer.

To prepare the TQ or Ultivo TQ mass spectrometer:

1. If you are using one computer to control the RapidFire instrument and the mass spectrometer, go to step 2.

   If you are using two computers to control the RapidFire instrument and the mass spectrometer:

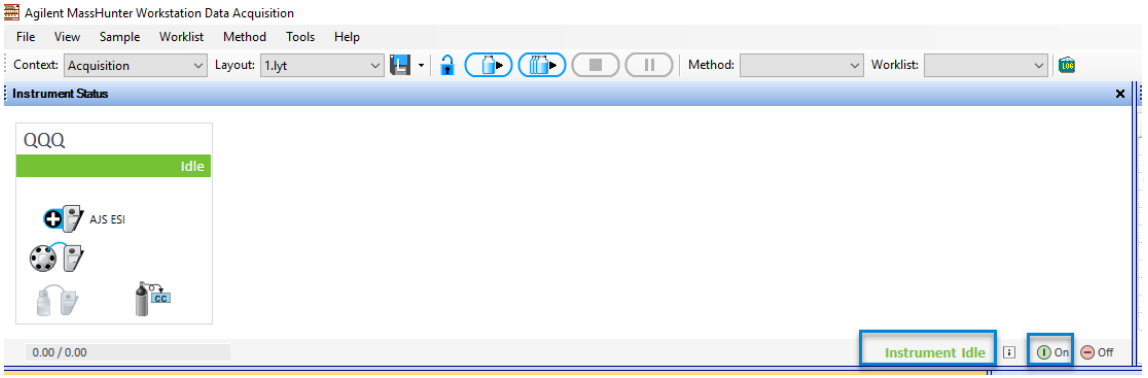
   a Open RapidFire Communicator by double-clicking the desktop icon ( ).

   b Open the Windows Task Manager and verify the following processes are running:

   - RFRemServer.exe
   - RFMasshunterS_TQ.exe

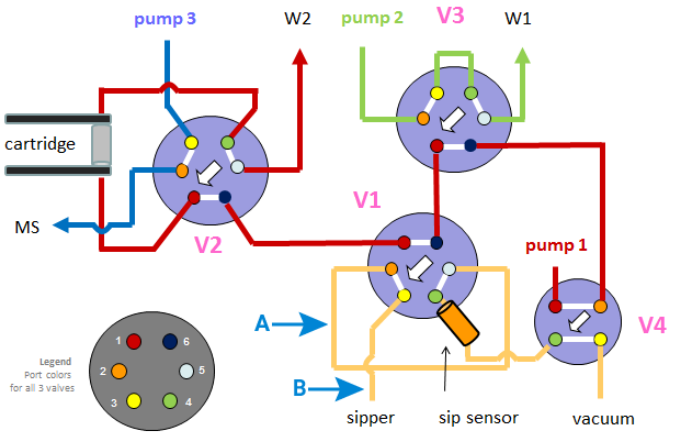
2. Start Mass Hunter Data Acquisition software by double-clicking the Data Acquisition desktop icon.
3. Turn on the detector by clicking **On** in the TQ device pane or in the status line for the Instrument Status area.

   The status indicator of the device in the **Status** area turns from teal ((Standby, connected), to yellow (Not Ready, preparing), and then to green (Ready, idle).



Minimizing the required sample volume

The volume used per sample well for the standard setup of the RapidFire 400 System is approximately 35 µL. This includes 10 µL of sample for measurement and 25 µL of dead volume, composed of a sample loop (A) and sipper tube (B).



It is possible to reduce the sample size to 5 µL and 10 µL with the use of smaller diameter sipper and sample loop tubing. The use of 1536-well microplates require smaller sample size because of the limited volume in each well.

The smaller diameter tubing and other required parts are available from Agilent Technologies. The required parts and the amount of sample that is measured are listed in the table below.

Parts for 10 µL sample measurement

Part number	Quantity	Description	Notes
RF0052T	1	0.5” guide needle	Replaces 1.5” guide needle
RF0111T-8	1	8” OF 0.009” ID tubing, gray	Cut to length (4.5” for sipper tube, 3.5” for sample loop)
RF0094T	1 (10 pk)	Ferrule, red od 1/32”	One per connection

Parts for 5 µL sample measurement

Part number	Quantity	Description	Notes
RF0052T	1	0.5” guide needle	Replaces 1.5” guide needle
RF0112T-13	1	12” of 0.005” id tubing, red	Cut to length (4.5” for sipper tube, 3.5” for sample loop)
RF0094T	1 (10 pk)	Ferrule, red od 1/32”	One per connection

To run the system with 5 µL and 10 µL sample volumes:

1. Prepare the RapidFire 400 system:
   - **a** Replace the standard guide needle with the 0.5” guide needle.
   - **b** Replace the standard sample loop tubing and sipper tubing with the desired smaller diameter loop and sipper tubing.
2. To account for the shorter guide needle, reteach the stage teachpoints using the appropriate plate type. For details, see **“Setting the stage teachpoints”** on page 18.
3. Using a standard analyte, empirically determine the optimal sip time for your system by inactivating the sip sensor and creating sample runs with sip times between 50-200 ms. The optimized sip time will be used for the Aspirate step of the RapidFire method.

   For more information on performing runs with minimal sample volumes, contact customer support at Agilent Technologies.

Shutting down the RapidFire system between runs

When you have completed your runs using the RapidFire system, shut down the system as follows.

NOTE

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

To shut down the system:

1. If your samples contain high-salt and/or high-protein:
   - **a** Flush the sipper to avoid the formation of precipitates as described in **"Preparing the sipper"** on page 52.
   - **b** Clean the sample flow path by running a minimum of 50 wells containing methanol.
2. Close the RapidFire Software:
   - **a** In the RapidFire software window, click **Disconnect** to disconnect the software from the instrument.
   - **b** Close the RapidFire software window.
   - **c** In the RapidFire Control Panel, click **Stop**.
   - **d** Close the RapidFire Control Panel.
3. Put the mass spectrometer into standby mode.
4. Turn off the RapidFire vacuum source. DO NOT turn off the mass spectrometer vacuum source.

3 Operating the RapidFire 400 in Plates mode

This section describes how to operate the RapidFire 400 in Plates mode. It contains the following topics:

About Plates mode 66
Workflow for using Plates Mode 66
Before you start 66
Recommendations for preparing the plates 67
Loading a single plate 68
Loading multiple plates using the plate handler 68
Preparing the TOF or Q-TOF mass spectrometer 70
Preparing the TQ or Ultivo TQ mass spectrometer 72
Setting up the RapidFire parameters for a Plates mode run 74
Starting a run in Plates mode 78
Pausing a run in Plates mode 79
Stopping a run in Plates mode 79
Maintaining a log book of data 80

About Plates mode

Plates mode can be used when you have one or more plates that you want to sample without a preset method that has a specific sipping sequence.

In Plates mode, you load your plate or plates and run them according to the conditions chosen in the RapidFire software window. The samples are fed into the mass spectrometer but the data collection is not synchronized to the RapidFire run.

This mode is primarily used for testing and optimizing conditions for data collection when using Sequence mode.

Workflow for using Plates Mode

Table 5. Plates Mode workflow steps

Step	Task	See...
1	Prepare the system	**"Before you start"** on page 66
1	Prepare plates	**"Recommendations for preparing the plates"** on page 67
2	Load plates	• **"Loading a single plate"** on page 68 • **"Loading multiple plates using the plate handler"** on page 68
3	Prepare MS	• **"Preparing the TOF or Q-TOF mass spectrometer"** on page 70 • **"Preparing the TQ or Ultivo TQ mass spectrometer"** on page 72
4	Set RF run parameters	**"Setting up the RapidFire parameters for a Plates mode run"** on page 74
5	Start run	**"Starting a run in Plates mode"** on page 78

Before you start

Ensure you have prepared the RapidFire system. Refer to the following topics:

- **"Starting the system"** on page 13
- **"Loading and homing the cartridge changer"** on page 16

- **"Minimizing the required sample volume"** on page 62, if you are running 1536-well plates or minimizing sample volume
- **"Homing the stage"** on page 18
- **"Checking the nanovalves"** on page 45
- **"Preparing the peristaltic pump"** on page 47
- **"Preparing the quaternary pumps"** on page 51
- **"Preparing the sipper"** on page 52.
- **"Preparing the temperature control unit"** on page 53

Recommendations for preparing the plates

- Ensure sample plates have thawed completely.
- Centrifuge the plates at 3000 rpm for at least five minutes.

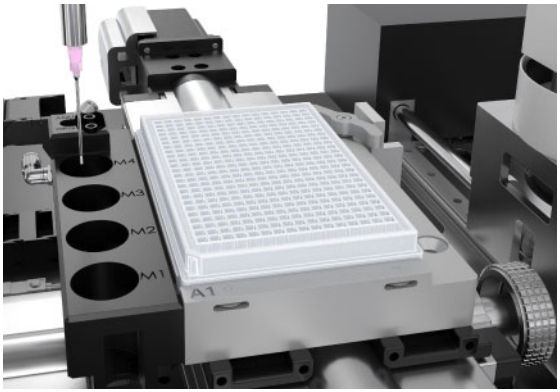
  Centrifuging the plates forces particles and insoluble material to the bottom of the wells where they are less likely to be aspirated into the system.

Loading a single plate

To load a single plate:

1 Ensure that the stage has been homed. See **"Homing the stage"** on page 18 for a procedure.

2 Open the doors and securely position the plate on the stage. Ensure well A1 is aligned with the A1 marking on the stage.



3 Close the doors.

Loading multiple plates using the plate handler

Before you start:

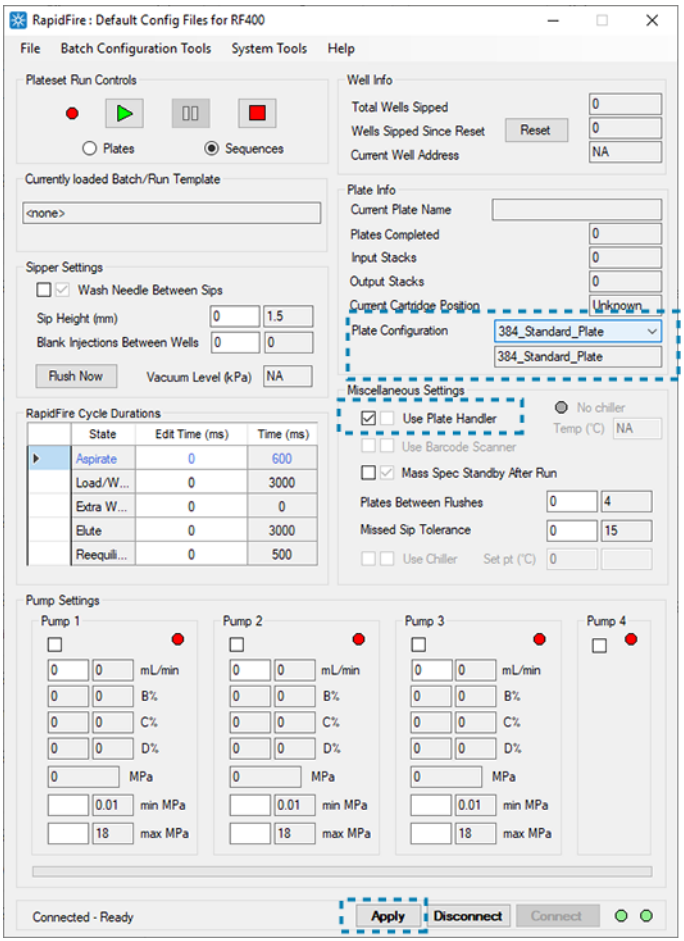
If you are using the temperature control unit, make sure it is on and has reached the set temperature. See **step 9** in **"Setting up the RapidFire parameters for a Plates mode run"** on page 74.

To load plates using the plate handler:

1 In the RapidFire software window:

   a Select the plate type you are using from the **Plate Configuration** list.

   b Select **Use Plate Handler** and click **Apply**.



2 Open the doors and pull out the drawer slide containing the three plate stacks.

3 Load the plates into two of the stacks. The A1 well should be oriented on the right-hand side towards the back of the unit as you are standing in front of the system and looking towards the back.

   Make sure one stack is empty so it can receive plates once they have been sampled.

Preparing the TOF or Q-TOF mass spectrometer

To prepare the TOF or Q-TOF mass spectrometer:

1 Set up the mass spectrometer as described in **"Preparing the TOF or Q-TOF mass spectrometer"** on page 53.

2 In the Sample Run window of the MassHunter Acquisition software:

   a Enter the **Name** and **Path** for the data file.

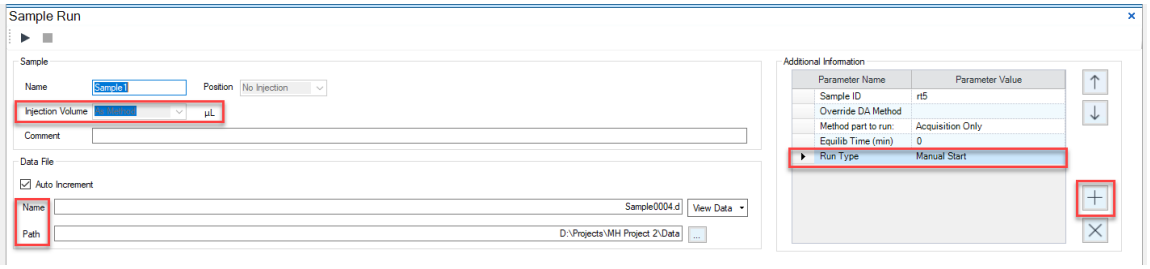
   The data file name must be **sequence1.d** (for data analysis by RapidFire Integrator).

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

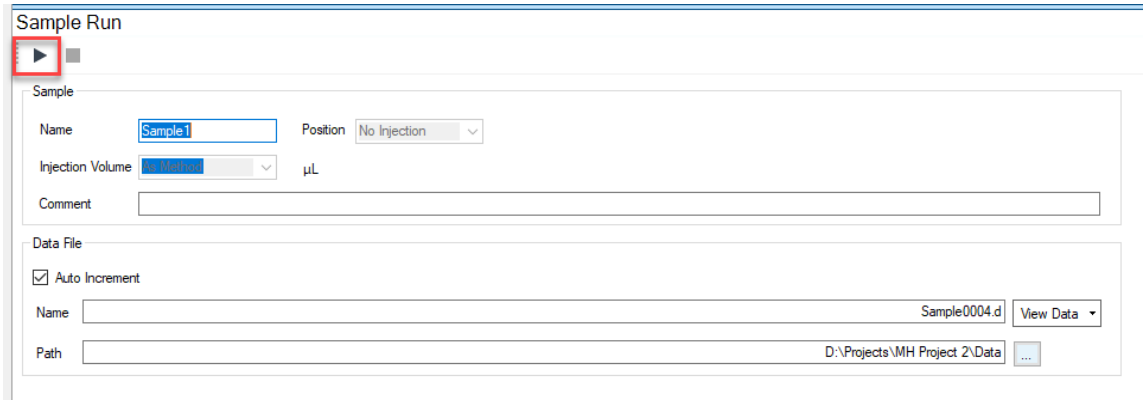
   c Select **RunType** and click **OK**.

   d For the **RunType**, 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 (line connected between port 2 and 5 on valve 1).



3 Click the start arrow (▶) to start the run.



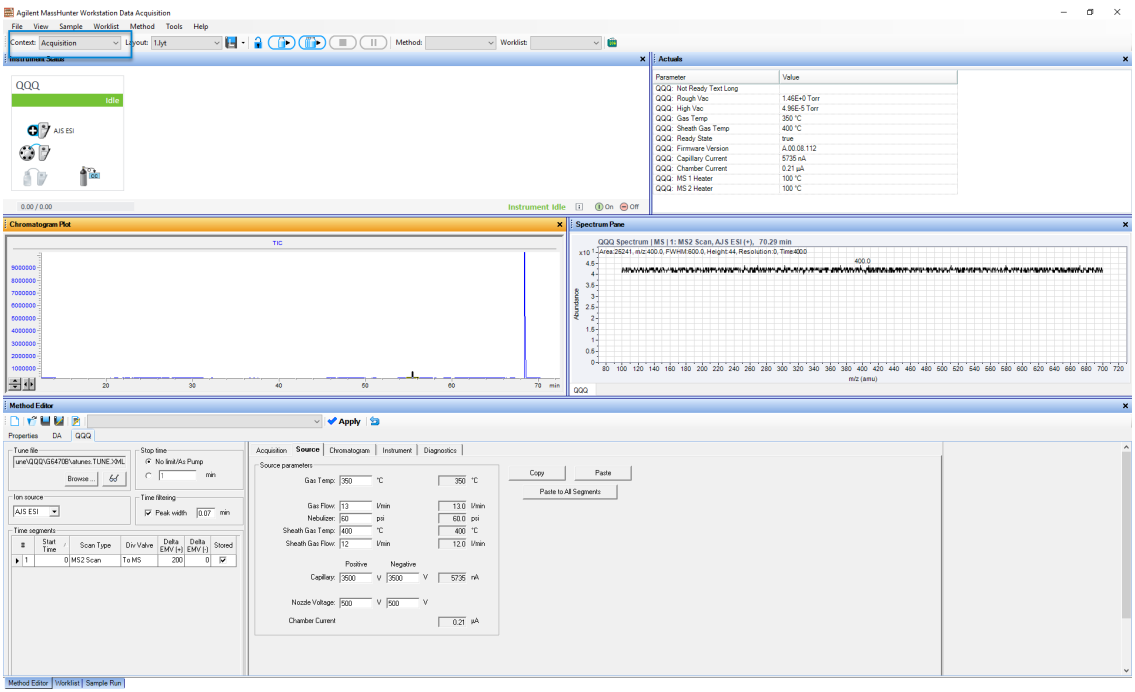
When the source has equilibrated, the statue indicator of the TOF or Q-TOF device module turns blue in the Instrument Status area.

Preparing the TQ or Ultivo TQ mass spectrometer

To prepare the TQ mass spectrometer:

1 Set up the mass spectrometer as described in **"Preparing the TQ or Ultivo TQ mass spectrometers"** on page 61.

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



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

Agilent recommends storing methods for the RapidFire system in the **D:\MassHunter\methods\RapidFire** folder.

4 Set the parameters for your analysis on the TQ tabs.

5 In 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 software).

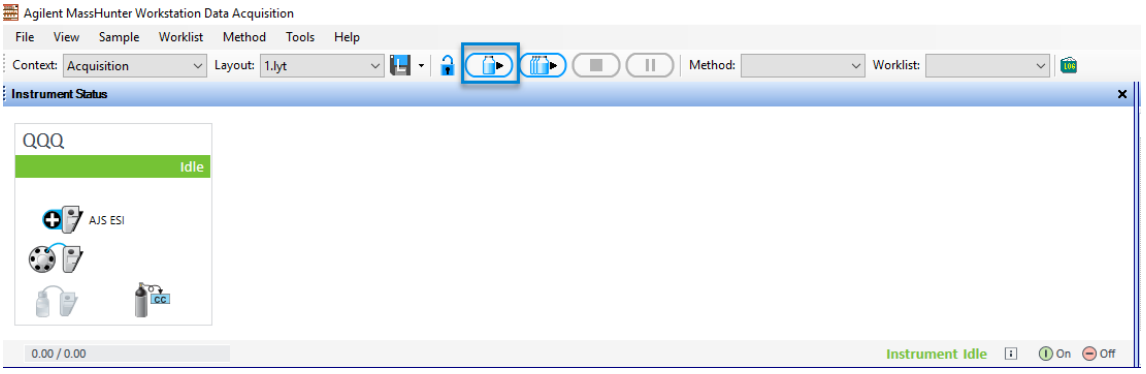
b Click to add a parameter to the **Additional Information** list.

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

d For the **RunType**, 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 **Start Sample Run**, or click **Sample > Run**.



When the source has equilibrated, the status indicator of the TQ device module turns blue in the Instrument Status area.

Setting up the RapidFire parameters for a Plates mode run

Before you start

Make sure that the cartridges are installed and the Cartridge Changer has been homed. See **"Loading and homing the cartridge changer"** on page 16 for a procedure.

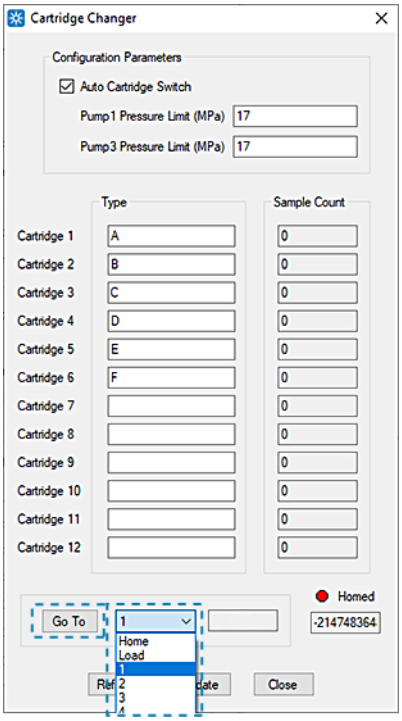
To set up the RapidFire run parameters for Plates mode:

1 In the RapidFire software window, click **System Tools > Cartridge Changer**.

The Cartridge Changer dialog box opens.

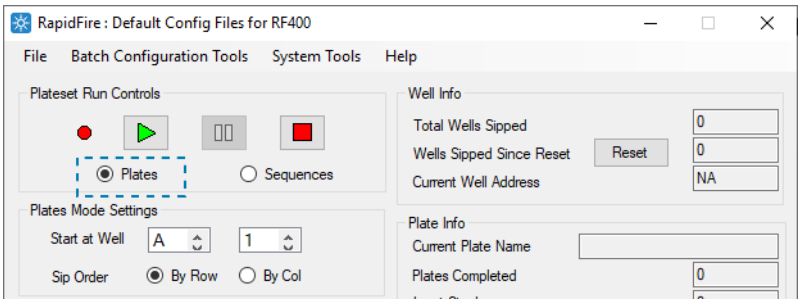
2 Select the position you want to use from the **Go To** list and click **Go To**.

Ensure the changer moves to the correct position the field to the right of the drop list should display the same number as shown in the Go To list.



3 Close the Cartridge Changer dialog box.

4 In the RapidFire software window, select **Plates** in the **Plateset Run Controls** area.



5 In the **Plates Mode Settings** area, set the following:

a Type the position of the well on the plate to start the run in **Start at Well** fields. The first position is A1.

b Select the order in which you want the run to proceed,

- **By Row** (for example, A1, A2, A3...H11, H12)
- **By Col** (for example, A1, B1, C1...G12, H12)

6 In the **Sipper Settings** area, set the following:

a Select the **Wash Needle Between Sips** check box to wash the sipper once after aspiration of the sample from each well. The sipper is washed first in the aqueous, and then in the organic wash chimney for 300 ms each.

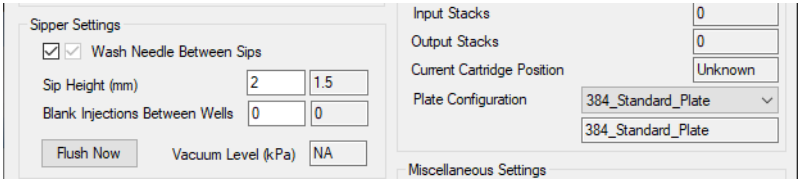
b In the **Sip Height** field, enter the number of millimeters (1 mm or more) from the lowest position of the sipper to the bottom of the well.

The sipper (in valve 1, port 3) travels down from the safe z position into the sample well until either:

- The sip sensor (in valce 1, port 4) optically detects the presence of fluid and trigger the actuation of valve 1, which causes the transition between State #1 (Aspiration) and State #2 (Load/Wash), or
- The z-position of the sipper reaches the set Sip Height.

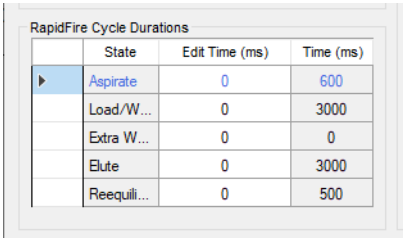
c If you want the system to do extra cycles in the organic wash station between samplings, enter a value (more than 0) for **Blank Injections Between Wells**.

This setting helps to limit or prevent carryover between samples.

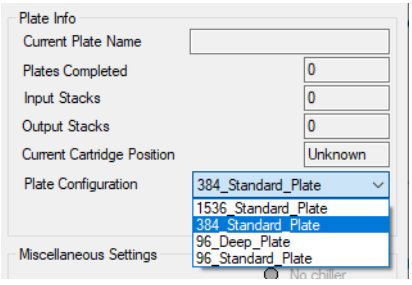


7 In the **RapidFire Cycle Durations** area, set the valve timing as per assay protocol for **Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, and **Reequilibrate**.

Set **Aspirate** to at least **600 ms** for reliable sip sensor triggering.



8 In the **Plate Info** area, select the plate type you are using from the **Plate Configuration** list.



9 In the **Miscellaneous Settings** area, do the following:

a Select the **Use Plate Handler** check box if you are using the plate handler to deliver the plates.

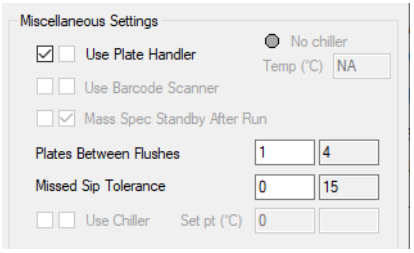
b Select the **Use Barcode Scanner** check box if you want to scan the plate barcode before the plate is loaded onto the stage.

c Do not select the **Mass Spec Standby After Run** check box. This option is not available in Plates mode.

d In the **Plates Between Flushes** field, enter the number of plates that should be sampled before the sipper is flushed for 1 min. Recommended values are **4** for a 96-well plate and **1** for a 384-well plate, but will depend on the specifics of a given method.

e In the **Missed Sips Tolerance** field, enter the number of times the sip sensor can miss detection of a sip before the system stops operation.

f *Systems with a temperature control unit*. Select the **Use Chiller** check box if you want to maintain a temperature below ambient during the run. Enter the temperature (°C) in the **Set pt** field that you want the unit to maintain during the run.



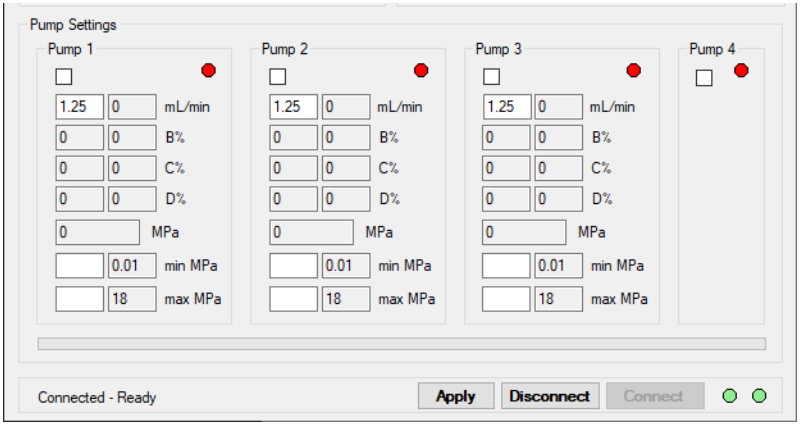
10 In the **Pump Settings** area do the following:

a Set pump flow rates for your run.

b Set the solvent compositions for your run.

c Set the minimum and maximum pressure for each pump.

11 Click **Apply**.



12 To save the parameter settings for future use, click **File > Save RF Method As** and enter a name for the method and click **Save**.

Starting a run in Plates mode

Before you start

- Initiate the mass spectrometer acquisition run before you start the RapidFire run. (Runs in Plates mode are not synchronized with the mass spectrometer.)
- Make sure the doors are closed and the emergency stop is in the released position.

To start a run in Plates mode:

1 Click start in the upper left area of the screen.

2 When prompted, enter the plate name or input the barcode using the hand-held barcode scanner and then click **OK**.

3 Verify that the sipper is aspirating the samples and the analytes are being detected by the mass spectrometer.

4 If you are loading plates manually, when the plate run is complete, you will be prompted to load a new plate. Do one of the following:

   a If you have more plates to run:

   - Open the doors and remove the previous plate.
   - Place your next plate on the stage as described in **"Loading a single plate"** on page 68 and close the doors.
   - Enter a name for the new plate or scan the plate barcode with the hand-held barcode scanner.

     If you do not enter a plate name within 30 minutes, a timeout automatically puts the RapidFire System in standby mode to prevent wasting solvent.

   b If you are done running plates, click **Cancel**.

Pausing a run in Plates mode

To pause a run:

1 Click pause .

   The system pauses after the cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Re-equilibrate**, and **Needle Wash** completes. The mass spectrometer continues to collect baseline data and the RapidFire pumps continue to run.

2 When you are ready to resume operation, click start .

   The system resumes operation where it left off and data is saved to the current data file.

Stopping a run in Plates mode

To stop a run that you do not want to restart again:

1 Click stop .

   The system stops after the cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Re-equilibrate**, and **Needle Wash** completes. Data acquisition stops and the current data file closes.

Maintaining a log book of data

Agilent recommends keeping a record of the data sets that are stored in the computer in order to retrieve the data more easily and match the Plates mode data sets of the RapidFire system and mass spectrometer in data analysis.

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

- **Date**
  Generated data is stored by date in the RapidFire computer. See the Data Analysis Guide for more information.
- **Assay description**
  What RF and MS methods were used.
- **Sample name**
  What the RF and MS data file names were used.
- **Set of plates number** as assigned by the RapidFire system.
  See the Data Analysis Guide for more information.
- **Plate number** (in the current set of plates)
- **Comments**

4 Operating the RapidFire 400 in Sequences mode

This chapter describes how to operate the RapidFire 400 system in Sequences mode. It contains the following topics:

About Sequences mode **82**
Workflow for operating in Sequences mode **83**
Before you start **84**
Creating a RapidFire Method file (.rfcfg) **84**
Creating a RapidFire Sequence PlateMap file (.rfcfg) **88**
Creating a RapidFire Batch file **90**
Recommendations for preparing the sample plates **95**
Loading a single plate **95**
Loading multiple plates **96**
Preparing the TOF or Q-TOF mass spectrometer **97**
Preparing the TQ or Ultivo TQ mass spectrometer **98**
Starting a run in Sequences mode **98**
Using an information management file for a Sequences mode run **99**
Creating a map file using MapFile Generator utility **100**
Starting the run in Sequences mode using an information management file **108**
Pausing a run in Sequences mode **110**
Stopping a run in Sequences mode **111**
Maintaining a log book of data **111**

About Sequences mode

Sequences mode can be used when you want to assay one or more sequences from sample wells in one or more plates. The plate or plates are run as a batch and the data collection by the mass spectrometer is synchronized with the RapidFire sample processing.

The following figure shows the structure of a batch and the files that make up a batch.

A batch file (.bat) consists of one or more plates. Each plate can have one or more sequences to be sampled. Each sequence is associated with a plate map (.rfmap) that defines the wells to sample. Each plate map is associated with an RF method (.rfcfg) and cartridge. The sample exiting the cartridge is ported directly to the mass spectrometer which analyzes the sample according to a preselected mass spectrometer method.

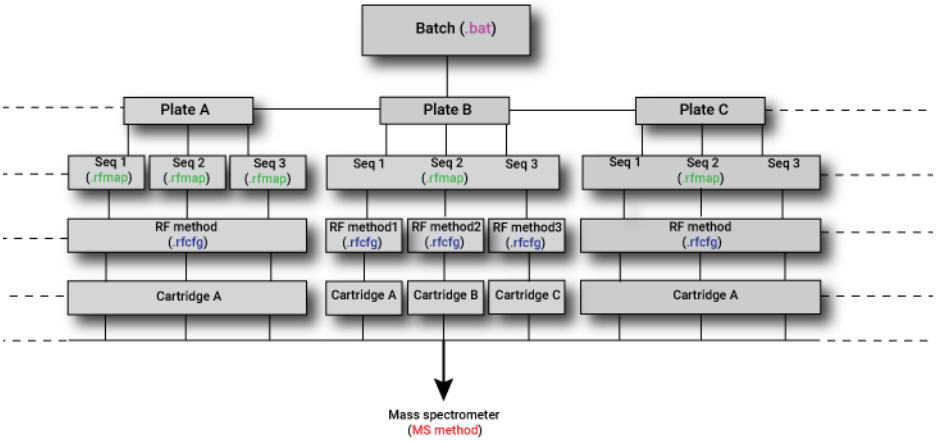


Figure 10. Structure of a batch file.

Workflow for operating in Sequences mode

Table 6. Workflow for Sequences mode

Step	Task	See
1	Prepare the system	**"Before you start"** on page 84
1	Create a method file	**"Creating a RapidFire Sequence PlateMap file (.rfcfg)"** on page 88
2	Create plate map file	**"Creating a RapidFire Sequence PlateMap file (.rfcfg)"** on page 88
3	Create batch file	**"Creating a RapidFire Batch file"** on page 90
4	Prepare plates	**"Recommendations for preparing the sample plates"** on page 95
5	Load plates	• **"Loading a single plate"** on page 95 • **"Loading multiple plates"** on page 96
6	Prepare MS	• **"Preparing the TOF or Q-TOF mass spectrometer"** on page 97 • **"Preparing the TQ or Ultivo TQ mass spectrometer"** on page 98
7	Start the run	**"Starting a run in Sequences mode"** on page 98
8	Pause the run	**"Pausing a run in Sequences mode"** on page 110
9	Stop the run	**"Stopping a run in Sequences mode"** on page 111

Before you start

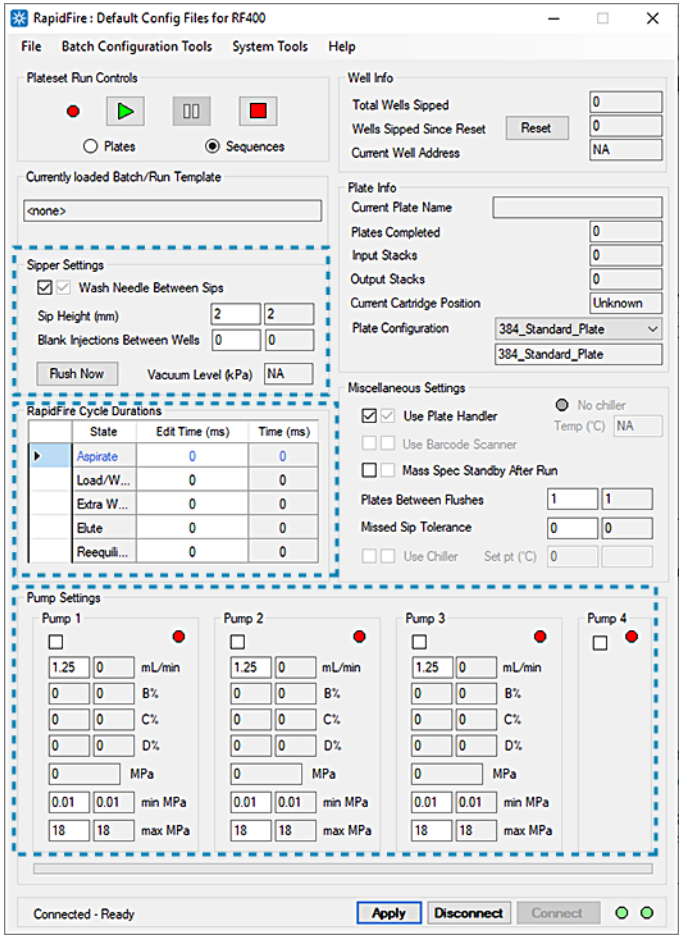
Ensure you have prepared the RapidFire system. Refer to the following topics:

- **"Starting the system"** on page 13
- **"Loading and homing the cartridge changer"** on page 16
- **"Minimizing the required sample volume"** on page 62, if you are running 1536-well plates or minimizing sample volumes
- **"Homing the stage"** on page 18
- **"Checking the nanovalves"** on page 45
- **"Preparing the peristaltic pump"** on page 47
- **"Preparing the quaternary pumps"** on page 51
- **"Preparing the sipper"** on page 52
- **"Preparing the temperature control unit"** on page 53

Creating a RapidFire Method file (.rfcfg)

Running the RapidFire in Sequences mode requires a RapidFire Method file (.rfcfg). You create the Method file by setting the parameters in the RapidFire UI main window and then saving them as an .rfcfg file. The Method file saves the parameter settings in the following areas:

- Sipper Settings
- RapidFire Cycle Durations
- Pump Settings



To set the parameters for the method:

1 In the **Sipper Settings** area, set the following:

a Select the **Wash Needle Between Sips** check box to wash the sipper once after aspiration of the sample from each well. The sipper is washed first in the aqueous, and then in the organic wash chimney for 300 ms each.

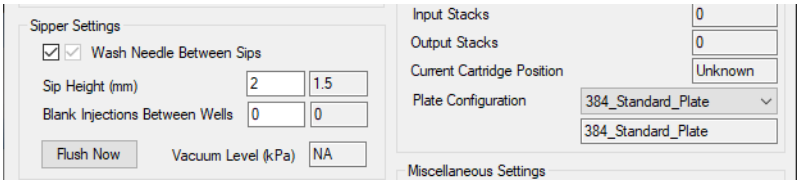
b In the **Sip Height** field, enter the number of millimeters (1 mm or more) from the lowest position of the sipper to the bottom of the well.

The sipper (in valve 1, port 3) travels down from the safe z position into the sample well until either:

- The sip sensor (in valve 1, port 4) optically detects the presence of fluid and triggers the actuation of valve 1, which causes the transition between State #1 (Aspiration) and State #2 (Load/Wash).
- The z-position of the sipper reaches the set Sip Height.

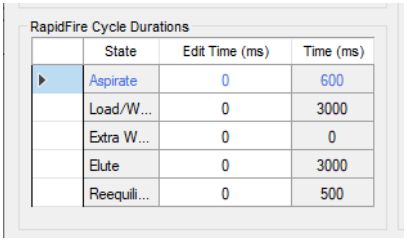
c If you want the system to do extra cycles in the organic wash station between samplings, enter a value (more than 0) for **Blank Injections Between Wells**.

This setting helps to limit or prevent carryover between samples.



2 In the **RapidFire Cycle Durations** area, set the valve timing as per assay protocol for **Aspirate**, **Load/Wash**, **Extra Wash**, **Elute**, and **Reequilibrate**.

Set **Aspirate** to at least **600 ms** for reliable sip sensor triggering.



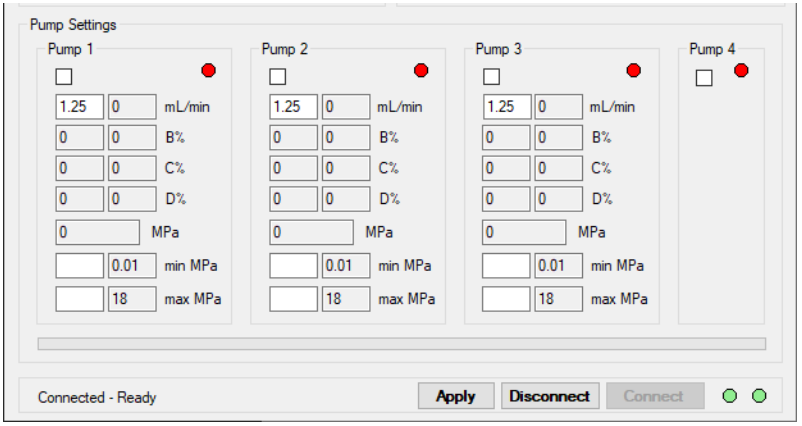
3 In the **Pump Settings** area do the following:

a Set pump flow rates for your run.

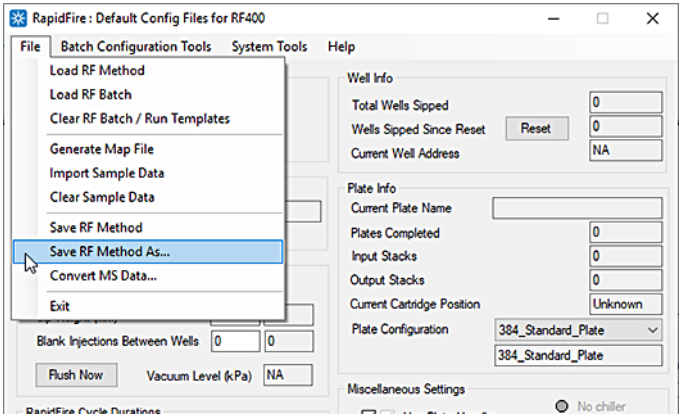
b Set the solvent compositions for your run.

c Set the minimum and maximum pressure for each pump.

4 Click **Apply**.



5 Click **File > Save RF Method As**, enter a name for the method and click **Save**.



Creating a RapidFire Sequence PlateMap file (.rfcfg)

Running the RapidFire system in Sequences mode requires a RapidFire Sequence PlateMap file (.rfmap).

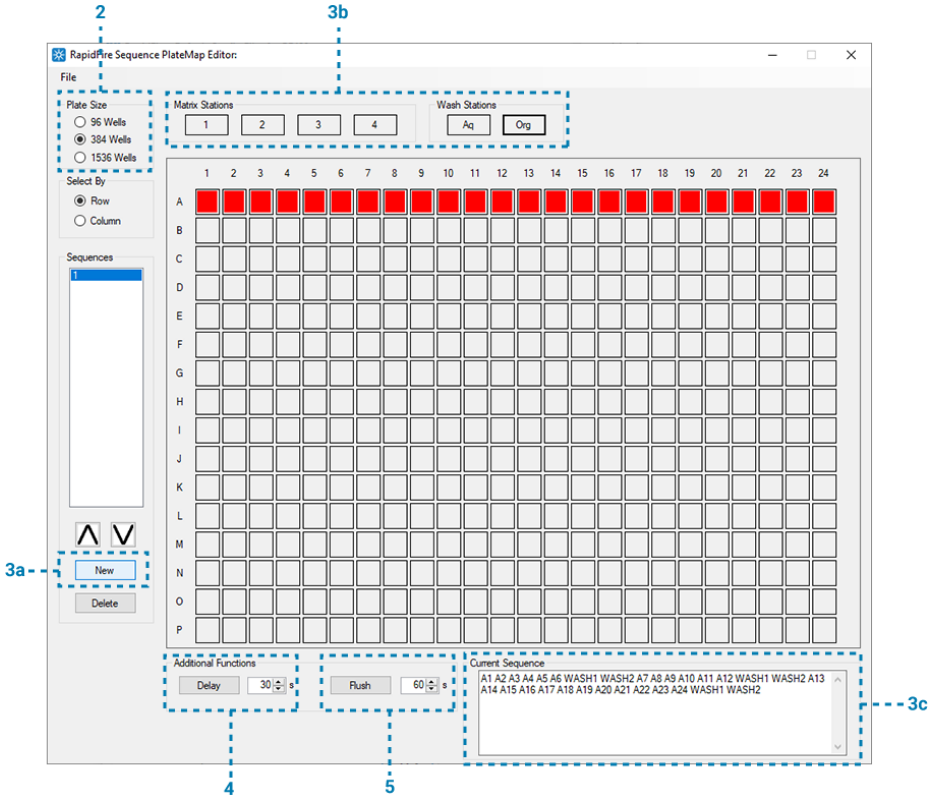
To create a RapidFire Sequence Platemap file:

1. In the RapidFire UI window, click **Batch Configuration Tools > Plate Map Editor**.
2. Select a **Plate Size**, 96-, 384-, or 1536-wells.
3. Create a Sequence:
   - **a** Click **New**.

     The number 1 appears in the **Sequences** field indicating this is the first sequence.
   - **b** Click on a well, **Matrix Station (1-4)**, or **Wash Station (Aqueous, Organic)** to add it to the sequence. To select multiple wells, click and drag the cursor over the wells you want to select.
   - **c** View the sequence in the **Current Sequence** box. You can edit the sequence in this area.
   - **d** To create another sequence, repeat this step.

NOTE

If you are running 1536-well plates with a TOF or QTOF mass spectrometer and acquiring large amounts of data by scanning at a slow rate or a wide mass range, the data file might become too large for the software to parse and analyze. To avoid this, you can create multiple sequences within a plate so that the data will be spread among multiple files.



4 *Optional*. You can enter a special well designation for each sequence to insert a pause in the RapidFire system data acquisition, as follows:

- **a** Place the cursor in the **Current Sequence** field at the point you want to pause, for example, at the end of a sequence.
- **b** Enter the time (in seconds) you want to delay the acquisition in the field next to **Delay**.
- **c** Click **Delay**.

5 *Optional*. You can enter a flush at a desired point in the sequence, for example, between sequences, as follows:

- **a** Place the cursor in the **Current Sequence** field at the point you want to flush.
- **b** Enter the time (in seconds) you want to flush the sipper in the field next to **Flush**.
- **c** Click **Flush**.

6 Click **File > Save As.** Enter a name for the Sequence PlateMap (.rfmap) and click **Save**.

Creating a RapidFire Batch file

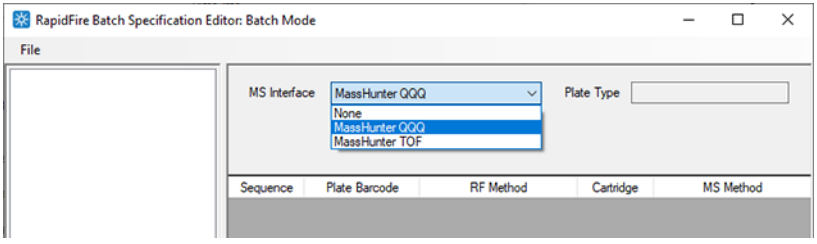
After you have created a method and plate map for the RapidFire system, you can create a batch file.

To create a batch file:

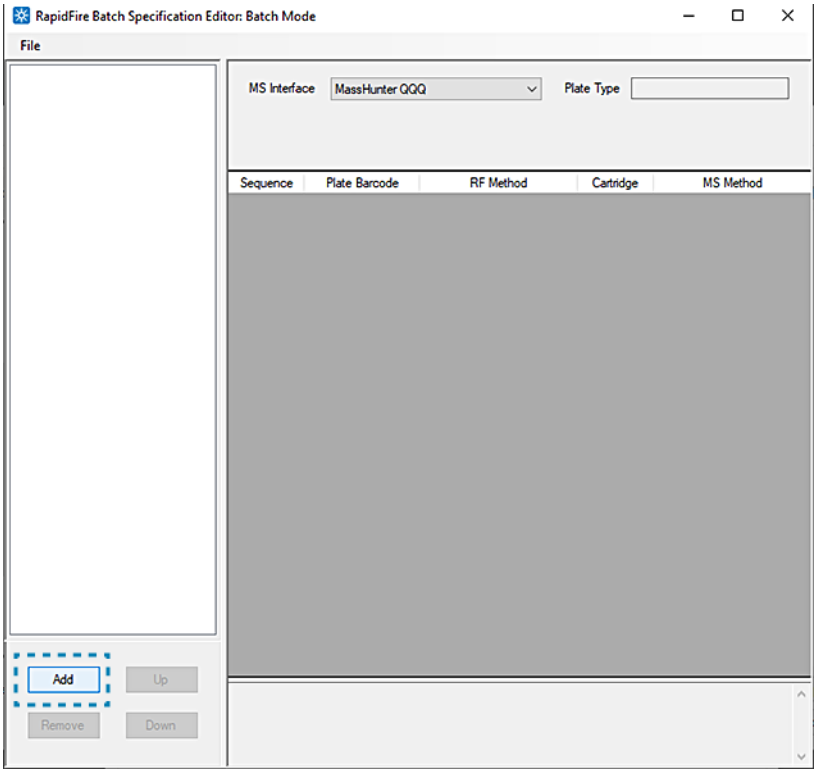
1 In the RapidFire software window, click **Batch Configuration Tools > Batch Editor**.

The Batch Editor dialog box opens.

2 Select the mass spec you are using from the **MS Interface** list.

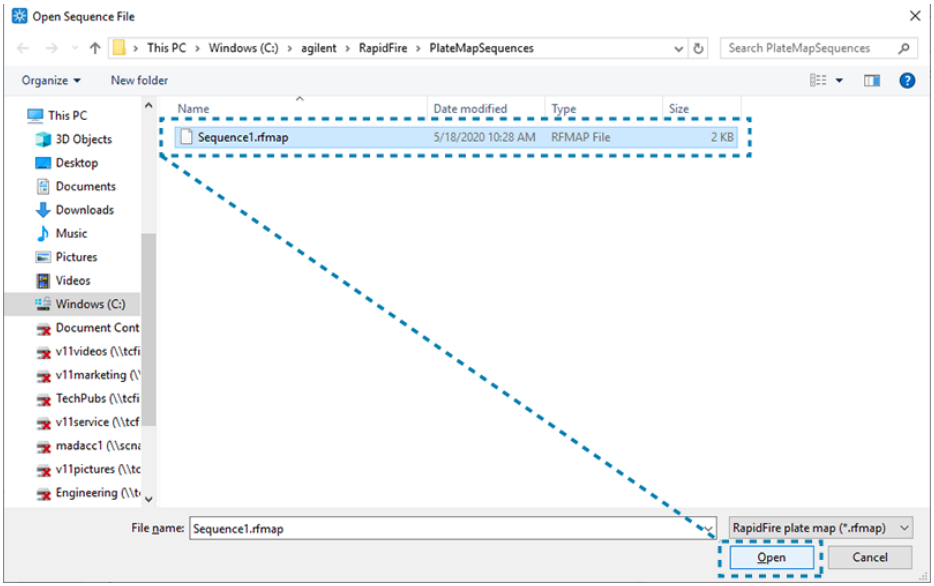


3 Click **Add**.

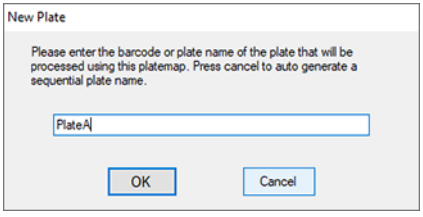


The Open Sequence File dialog box opens.

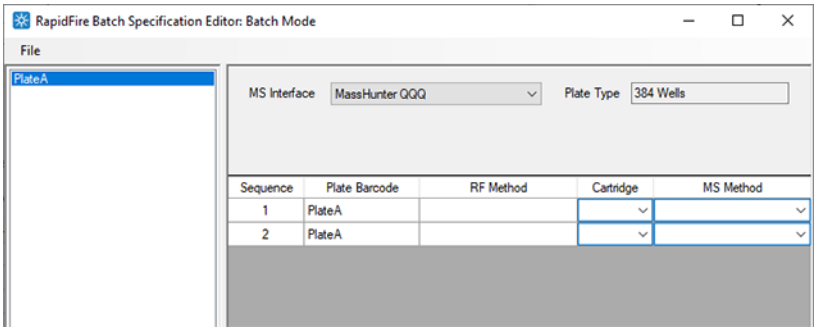
4 Select the sequence file (.rfmap) you want to use and click **Open**.



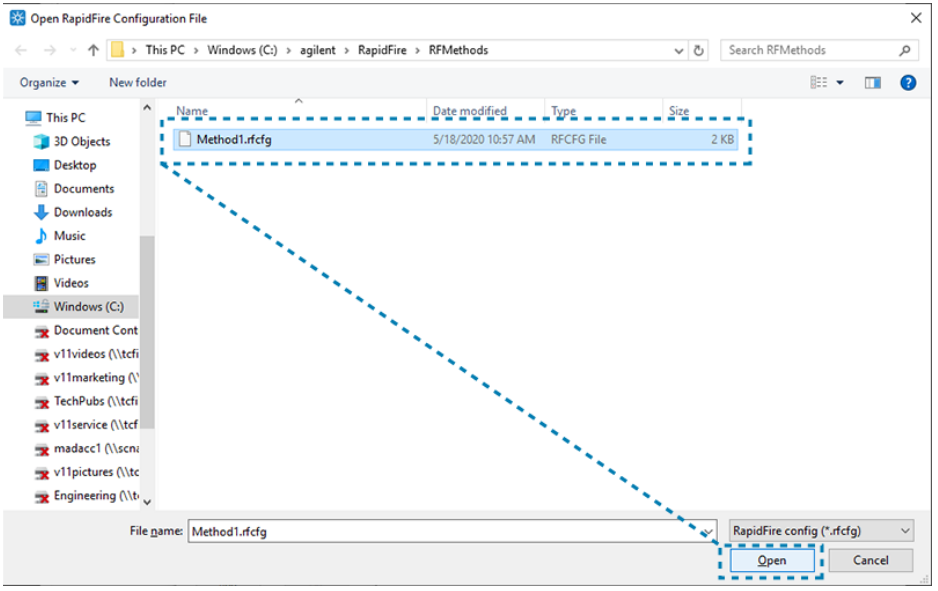
5 In the **New Plate** dialog box, enter the barcode or name of the plate that will be processed and click **OK**.



The Batch Editor dialog box displays the sequences identified in the plate map.



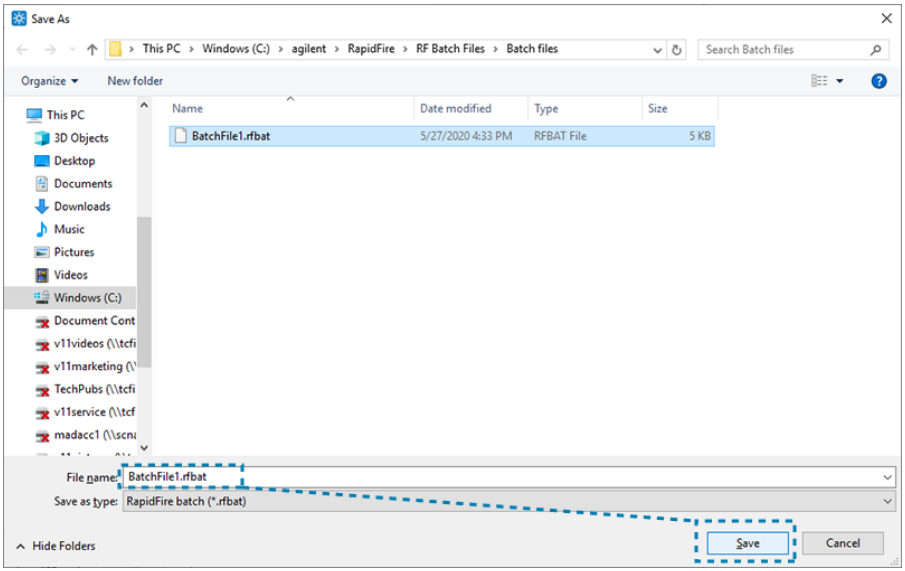
6 For each sequence, double-click in the **RF Method** field to open the Open File dialog box, select the RapidFire method (**.rfcfg**) file you want to use, and click **Open**.



7 For each sequence, in the **Cartridge** field, select the cartridge to use from the list.

8 For each sequence, in the **MS Method** field, select the MS Method to use from the list. (The methods should be located in D:\MassHunter\methods\RapidFire.)

9 Click **File > Save As**.



10 Enter a name for the batch file and click **Save**.

Recommendations for preparing the sample plates

- Completely thaw the sample plates.
- Centrifuge the plates at 3000 rpm for at least five minutes.

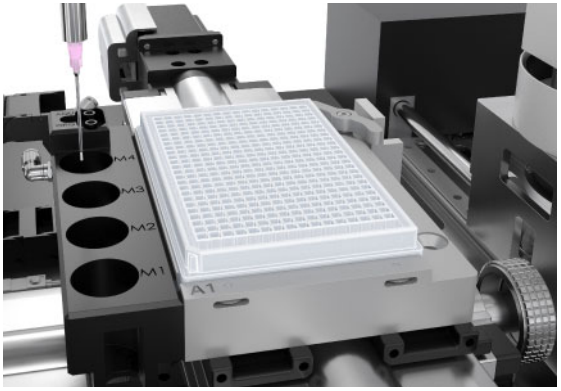
  Centrifuging the plates forces particles and insoluble material to the bottom of the wells where they are less likely to be aspirated into the system.

Loading a single plate

To load a single plate:

1 Ensure that the stage has been homed. See **"Homing the stage"** on page 18.

2 Open the doors and securely position the plate on the stage.



3 Close the doors.

Loading multiple plates

Before you start

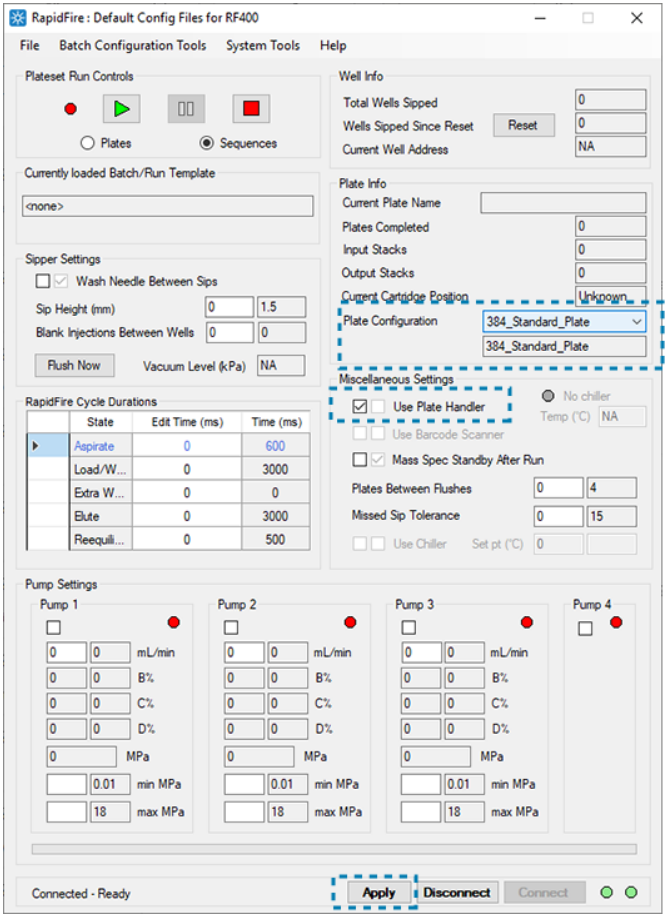
If you are using the temperature control unit, make sure the unit is on and the unit has reached the set temperature.

To load multiple plates:

1 In the RapidFire software window:

   a Select the plate type you are using from the **Plate Configuration** list.

   b Select **Use Plate Handler** and click **Apply**.



2 Open the doors and pull out the slide drawer containing the three plate stacks.

3 Load the plates into two of the stacks. The A1 well should be oriented on the right-hand side towards the back of the unit as you are standing in front of the system and looking towards the back.

   Make sure one stack is empty so it can receive plates once they have been sampled.

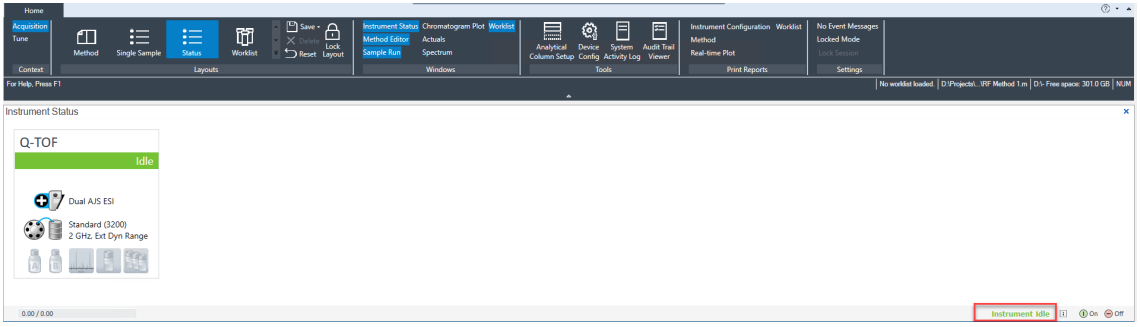
4 Close the drawer.

Preparing the TOF or Q-TOF mass spectrometer

To prepare the TOF or Q-TOF mass spectrometer:

1 Set up the mass spectrometer as described in **"Preparing the TOF or Q-TOF mass spectrometer"** on page 53.

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



In Sequences mode, the RapidFire system and MS system are synchronized and the data is recorded automatically in **D\MassHunter\Data\RapidFire**. Refer to the *Data Analysis Guide* for more information.

Preparing the TQ or Ultivo TQ mass spectrometer

To prepare the TQ mass spectrometer:

1 Set up the mass spectrometer as described in **"Preparing the TQ or Ultivo TQ mass spectrometers"** on page 61.

2 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.

Starting a run in Sequences mode

Before you start

1 In the RapidFire UI window, click **File > Load RF batch** and select the batch file (.rfbat) you want to run and click **Open**.

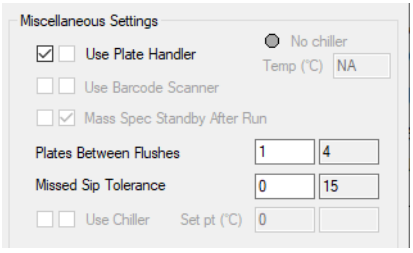
2 Set the parameters in the **Miscellaneous Settings** area:

   a Select the **Use Barcode Scanner** check box if you are using the system barcode scanner.

   b In the **Plates Between Flushes** field, enter the number of plates to run before flushing the system.

   c In the **Missed Sip Tolerance** field, enter the number of missed sips to allow before triggering an error.

   d Click **Apply** at the bottom of the window.



3 Make sure the doors are closed and the emergency stop is in the released position.

To start the run in Sequences mode:

1 Click start in the upper left area of the screen.

2 When prompted, enter the plate name or input the barcode using the hand-held barcode scanner and then click **OK**.

3 Verify that the sipper is aspirating the samples and the analytes are being detected by the mass spectrometer.

4 If you are loading plates manually, when the plate run is complete, you will be prompted to load a new plate. Do one of the following:

a If you have more plates to run:

- Open the doors and remove the previous plate.
- Place your next plate on the stage as described in **"Loading a single plate"** on page 95 and close the doors.
- Enter a name for the new plate or scan the plate's barcode with the hand-held barcode scanner.

  If you do not enter a plate name within 30 minutes, a time-out automatically puts the RapidFire System in standby mode to prevent wasting solvent.

b If you are done running plates, click **Cancel**.

Using an information management file for a Sequences mode run

You can use the MapFile Generator Utility to map your sample data columns from an information management file, for example LIMS, to a batch file (.bat) created in the RapidFire software. Once you create a map file, you import your sample data file before running a batch file.

Workflow

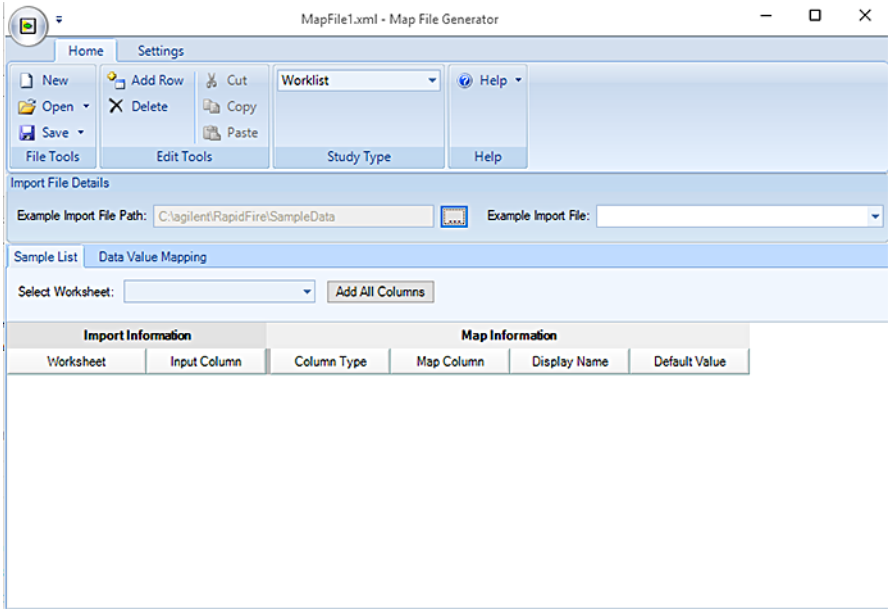
Step	For this task...	See...
1	Create a batch file	**"Creating a RapidFire Batch file"** on page 90
2	Create a map file	**"Creating a map file using MapFile Generator utility"** on page 100
3	Start the run	**"Starting the run in Sequences mode using an information management file"** on page 108

Creating a map file using MapFile Generator utility

To create a map file:

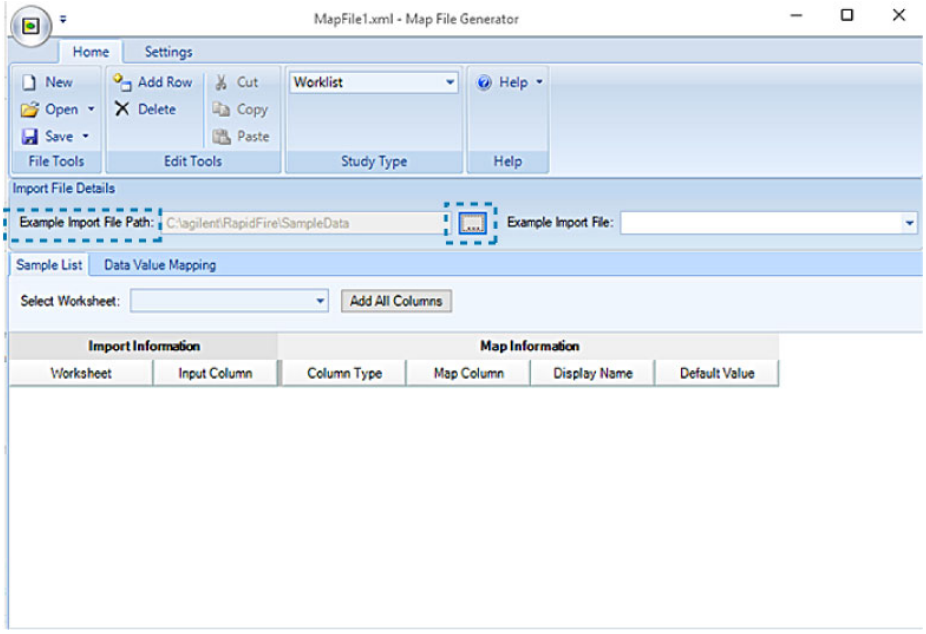
1 In the RapidFire UI window, click **File > Generate Map file**.

The Map File Generator dialog box opens.

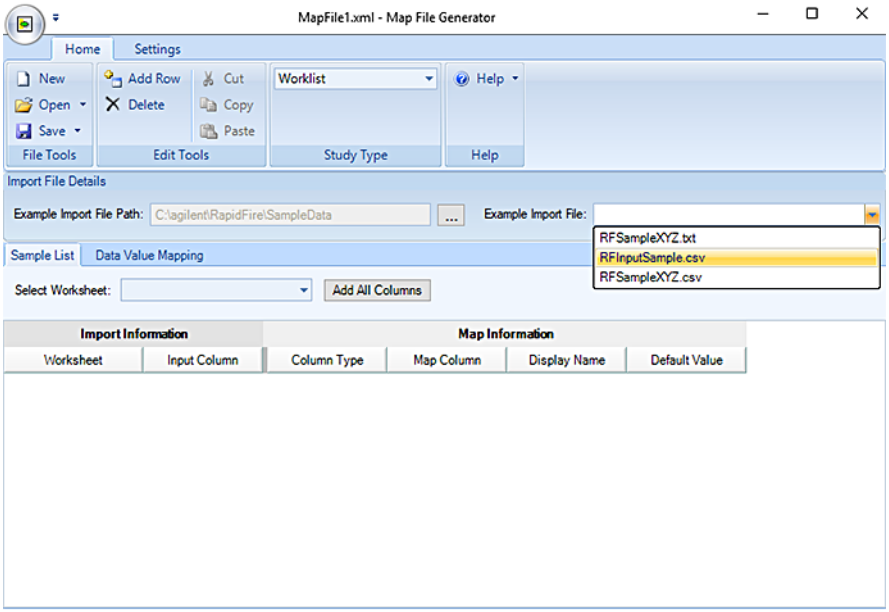


2 Click the browse button (...) next to the **Example Import File Path** field and select the location of the folder containing the sample file you wish to map.

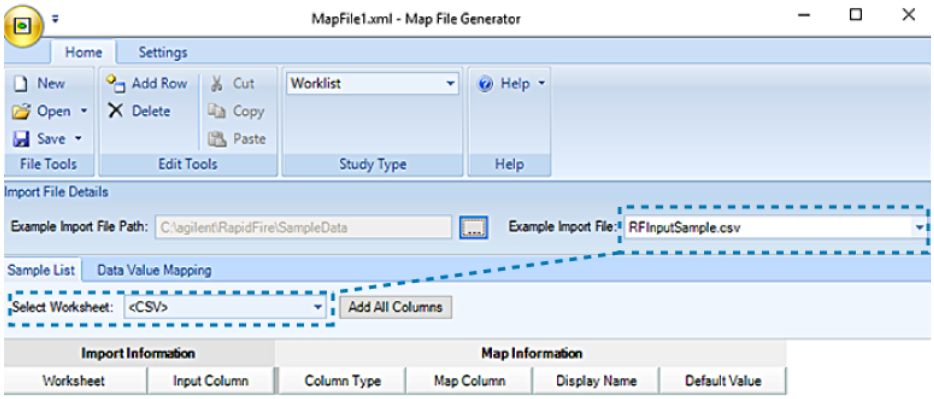
Once selected, the files within this folder are accessible from the **Example Import File** field.



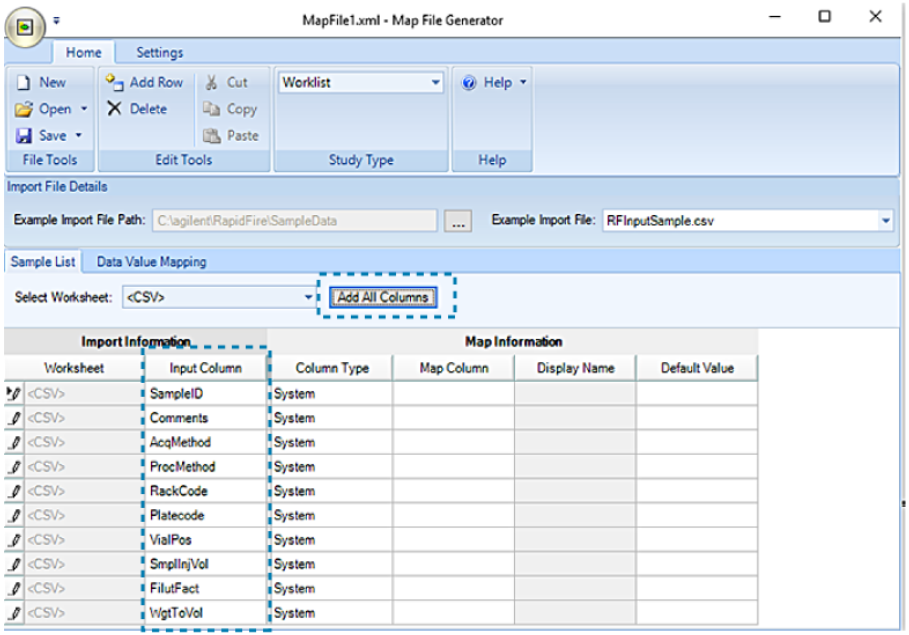
3 Select the specific file you want to map from the **Example Import File** list.



The Select Worksheet field displays the file type if the Example Import File is either a .txt or .csv file type. If the Example Import File is an Excel file (.xlsx), then the Select Worksheet field displays all the sheets in the Excel file.

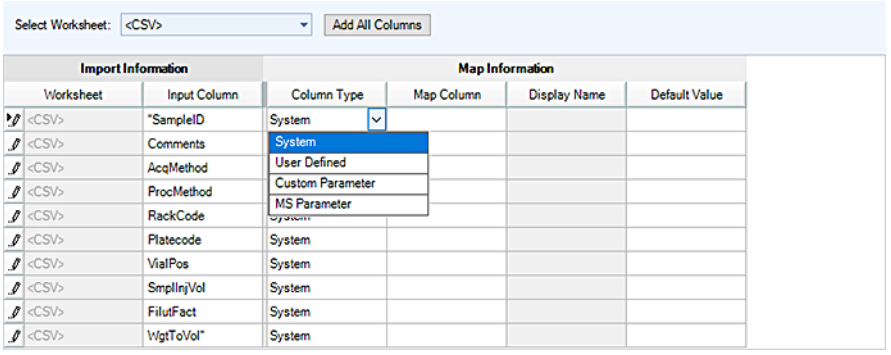


4 Click **Add All Columns** to display the column information from the input file.



5 Map the headings of the input column to the RapidFire column names:

a Select **System** for the **Column Type** for all input columns.



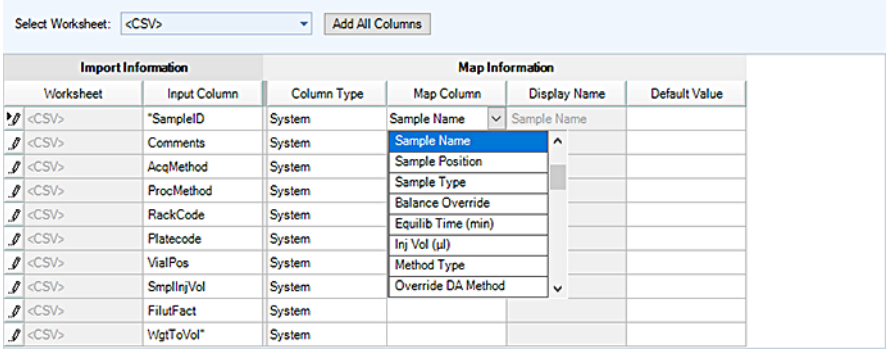
b Select a heading from the **Map Column** list that best corresponds to the heading in the input file.

For the import sample data feature to work correctly, make sure:

- The input data file has columns containing information of Sample Position and Barcode and the Sample Position is in the format A1, A2 and so forth.

- The index of columns in the sample input file for Sample Position, Barcode, and Run Template match the index of the corresponding fields in the map file.

  For example, if the input file has the Plate Position as the eighth column, then the Plate Position column must also be in the eighth position in the map file.

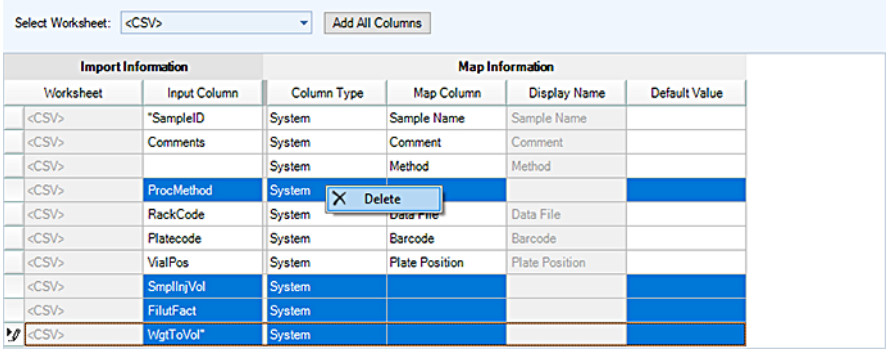


6 When you have matched all the input columns of interest, you can delete the ones not used.

To delete a column header:

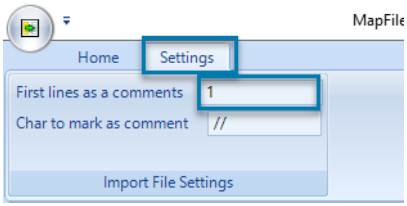
a Select the row containing the column header.

b Right-click and select **Delete**.



7 Set the data start line for the input file:

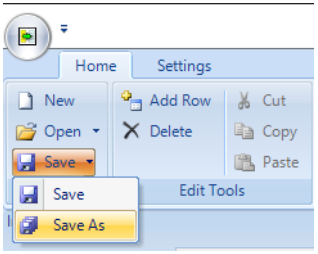
a Click **Settings** at the top of the window.



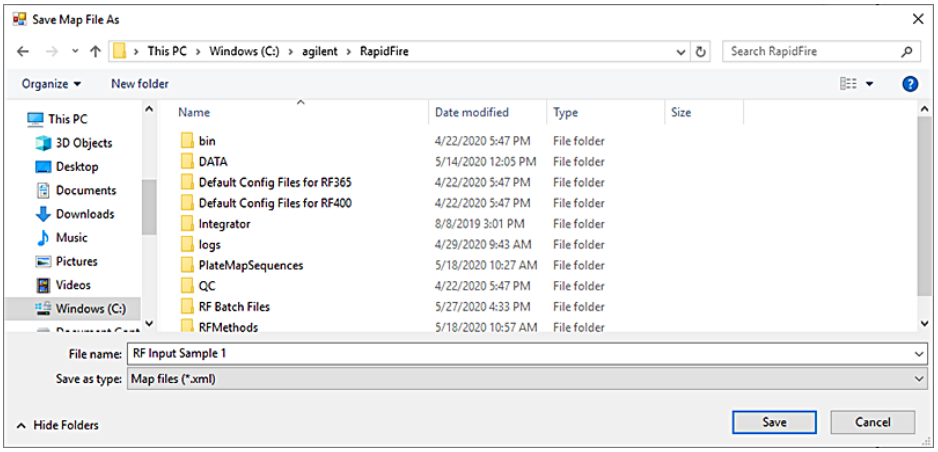
b In the **First lines as a comments** field, enter the number of lines that occur before the start of the actual data, including the header line, but not any comment lines.

For example, if you have no comment lines and no -extra- lines before the column header line, then enter 1 in this field.This represents the one line describing the column headers.

8 Click **Home** and then click **Save** and select **Save As** to open the Save file dialog box.



9 Select a location to save the file, type a name in the **File name** field, ensure the **Save as type** is **Map Files.xml**, and click **Save**.



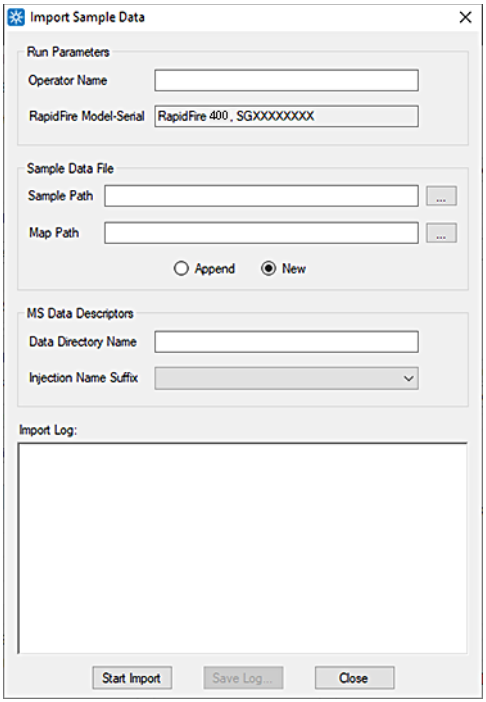
10 Close the Map File Generator utility.

Starting the run in Sequences mode using an information management file

To start the run:

1 In the RapidFire UI main window, click **File > Load RF Batch** and open the batch file you want to run using the sample data file you mapped in the previous procedure.

2 Click **File > Import Sample Data**. The Import Sample Data dialog box opens.



3 Fill in the data fields according to the following table:

Table 7. Import Sample Data dialog box parameters:

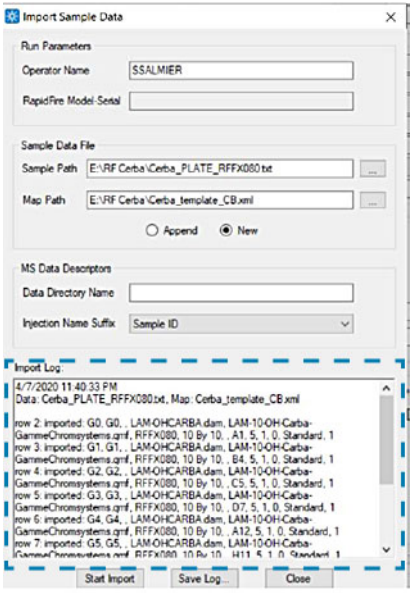
Parameter	Description/Action
Operator Name	*Optional*. Type in a name.
Sample Path	Source file to be analyzed. Click the Browse button and select the sample input file you used in the mapping procedure.

Table 7. Import Sample Data dialog box parameters:

Parameter	Description/Action
Map Path	Map file to use to match the columns of the sample input file to the Mass Hunter Quant columns. Click the Browse button and select the file.
Append	Select this option if this sample import is a continuation of a series of plates
New	Select this option if this is the first imported sample data file
Data Directory Name	Name of the directory where the MS data files will be stored. If left blank, the name will follow the convention year/month/day/number of plates analyzed.
Injection name suffix	Suffix added to the data file name. To activate the list, click **Start Import** and then select the desired suffix from the list.

4 If you have additional plate files, repeat **step 3** and be sure you select the **Append** option.

The Import Log area lists all the wells for each file of the imported plate in the order in which they were imported.

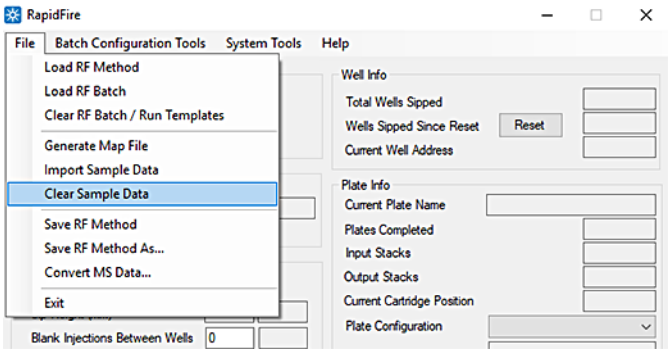


5 Click **Close** and start the run by clicking the start button ( ).

NOTE

Once the data is acquired and converted, the sample information will be retained when opening the data in MassHunter Analysis software.

6 When the run is finished, in the RapidFire software window, click **File > Clear Sample Data** before starting a new import sequence.



Pausing a run in Sequences mode

To pause a run in Sequences mode:

1 Click pause .

The system pauses after the cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Reequilibrate**, and **Needle Wash** completes. The mass spectrometer continues to collect baseline data and the RapidFire pumps continue to run.

2 When you are ready to resume operation, click start .

The system resumes operation where it left off and data is saved to the current data file.

Stopping a run in Sequences mode

To stop a run in Sequences mode:

1 Click stop ■ .

The system stops after the cycle of **Aspirate**, **Load/Wash**, **Extra Wash**, **Re-equilibrate**, and **Needle Wash** completes. Data acquisition stops and the current data file closes.

Maintaining a log book of data

Agilent recommends keeping a record of the data sets that are stored in the computer in order to retrieve the data more easily and match the data sets of the RapidFire and mass spectrometer in Data Analysis.

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

- **Date**
  Generated data is stored by date in the RapidFire computer. See the Data Analysis Guide for more information.
- **Assay description**
  What RF and MS methods were used.
- **Sample name**
  What the RF and MS data file names were used.
- **Set of plates number** as assigned by the RapidFire system.
  See the Data Analysis Guide for more information.
- **Plate number** (in the current set of plates)
- **Comments**

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5 Maintenance

This section describes the maintenance requirements for the RapidFire 400 System and how to shut the system down. This section contains the following topics:

Daily maintenance **114**
Weekly maintenance **114**
Shutting down the RapidFire system completely **114**

Daily maintenance

To maintain the system daily:

1 Flush the sipper as described in **"Preparing the sipper"** on page 52.

2 Do the shut down procedures described in **"Shutting down the RapidFire system completely"** on page 114.

Weekly maintenance

To maintain the system weekly:

1 Empty the waste containers, carboy and vacuum waste container in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

2 Clean the mass spectrometer according to the documentation for the mass spectrometer.

Shutting down the RapidFire system completely

To shut down the system completely:

3 Click **Disconnect** in the RapidFire software main window to disconnect the software from the instrument.

4 Close the RapidFire software main window.

5 Click **Stop** in the RapidFire Control Panel.

6 Close the RapidFire Control Panel.

7 If the RapidFire system and the mass spectrometer are being controlled by one computer, close the MassHunter Acquisition software.

8 Turn off the system by pressing the power switches on the back of the RapidFire system (NOT the power to the mass spectrometer).

9 Turn off the vacuum source for the RapidFire system (NOT the vacuum for the mass spectrometer).

10 Follow the mass spectrometer documentation instructions for shutting down the mass spectrometer.

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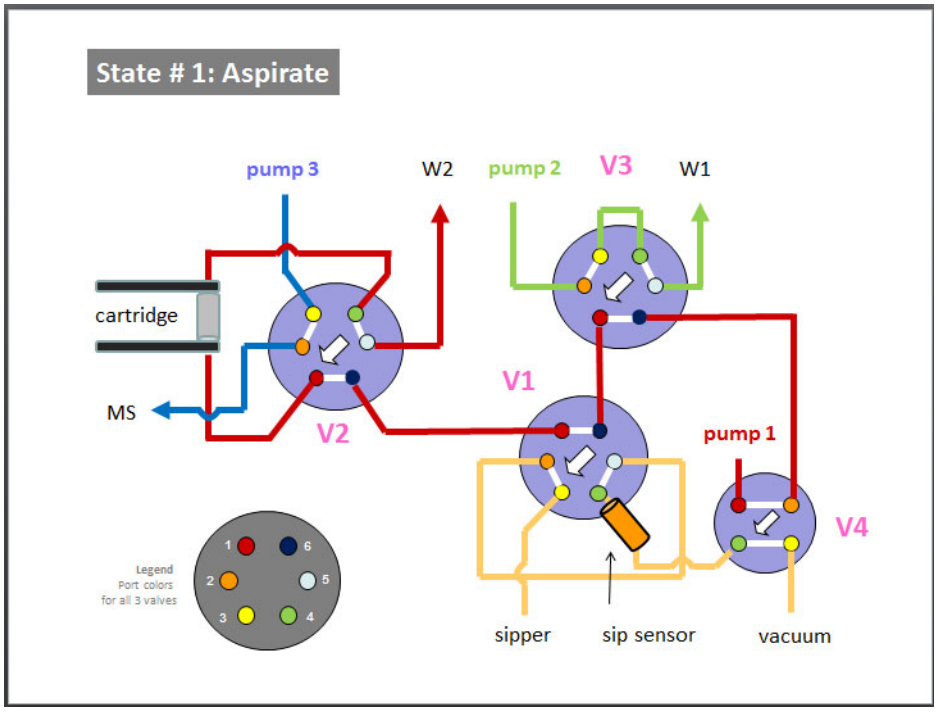
6 RapidFire System flow path reference

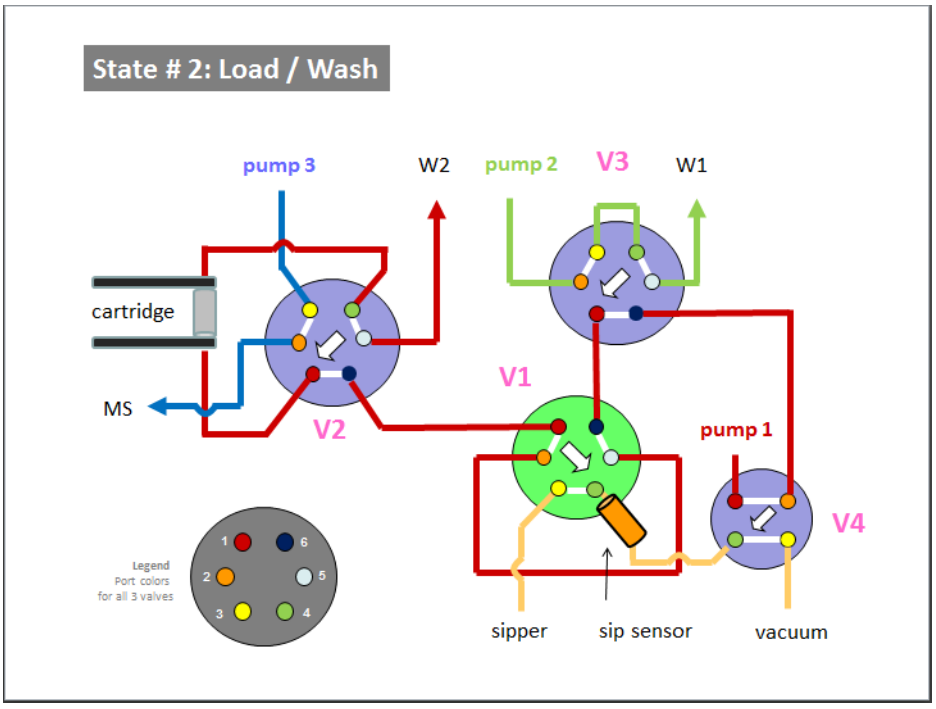
This sections provides figures that describes the flow paths for the various states of the nanovalves. This section contains the following topics:

State 1: Aspirate flow path **118**
State 2: Wash/Load flow path **119**
State 3: Extra Wash flow path **120**
State 4: Elute flow path **121**
State 5: Re-equilibrate flow path **122**
Flush the sipper flow path **123**
Physical colors of the tubing **124**
Valve positions **124**

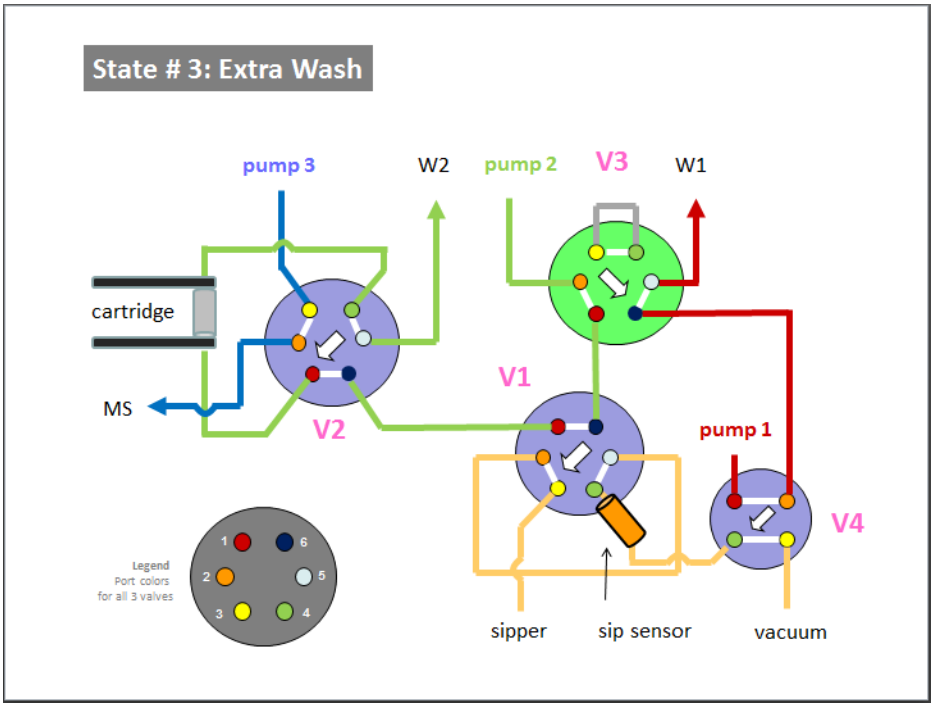
State 1: Aspirate flow path



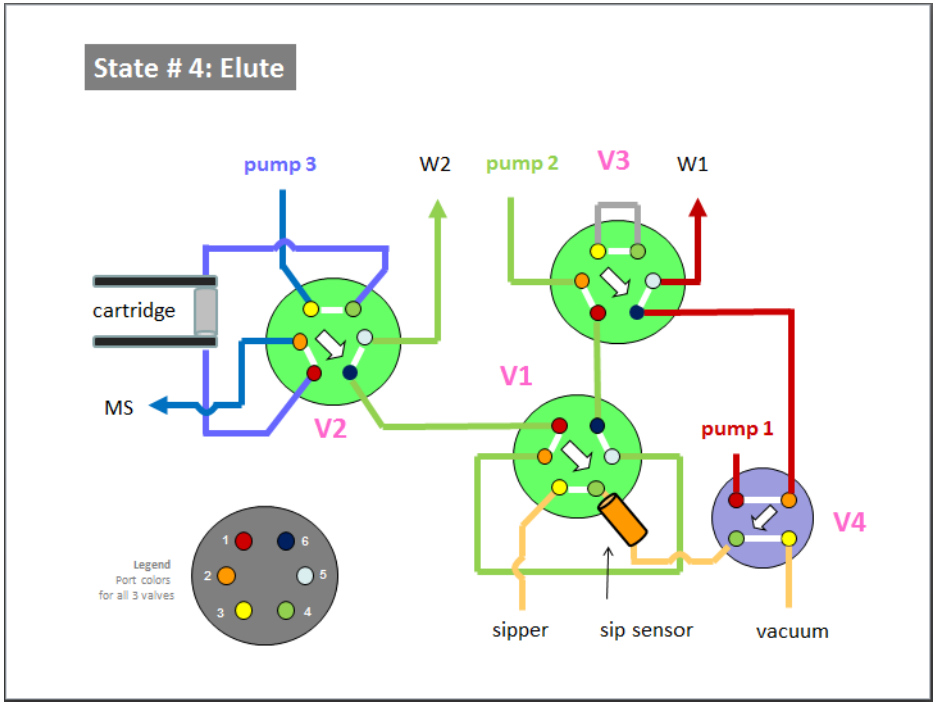
State 2: Wash/Load flow path



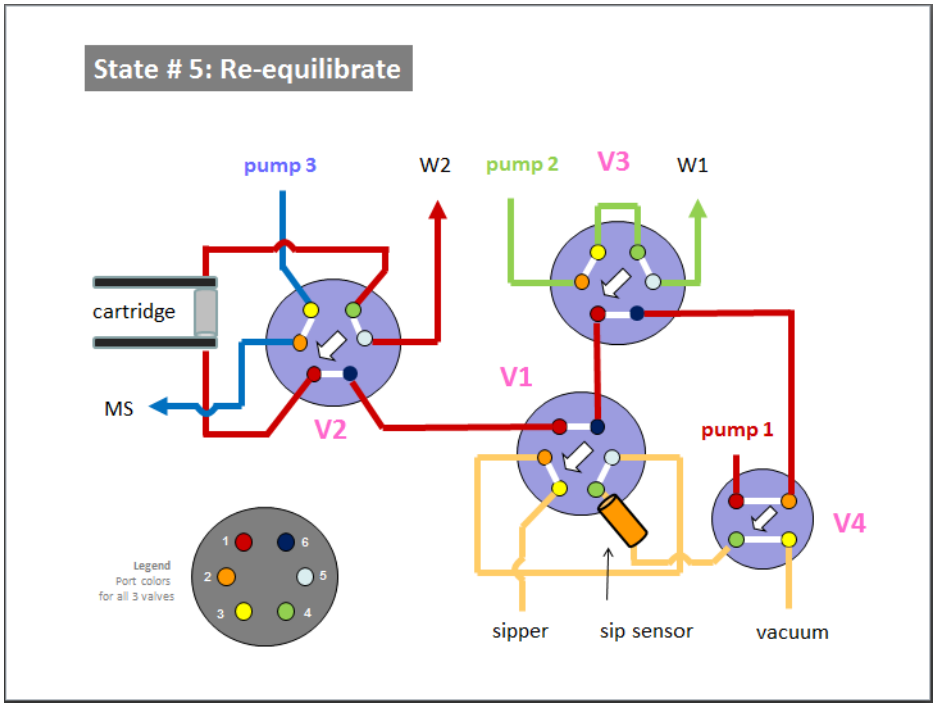
State 3: Extra Wash flow path



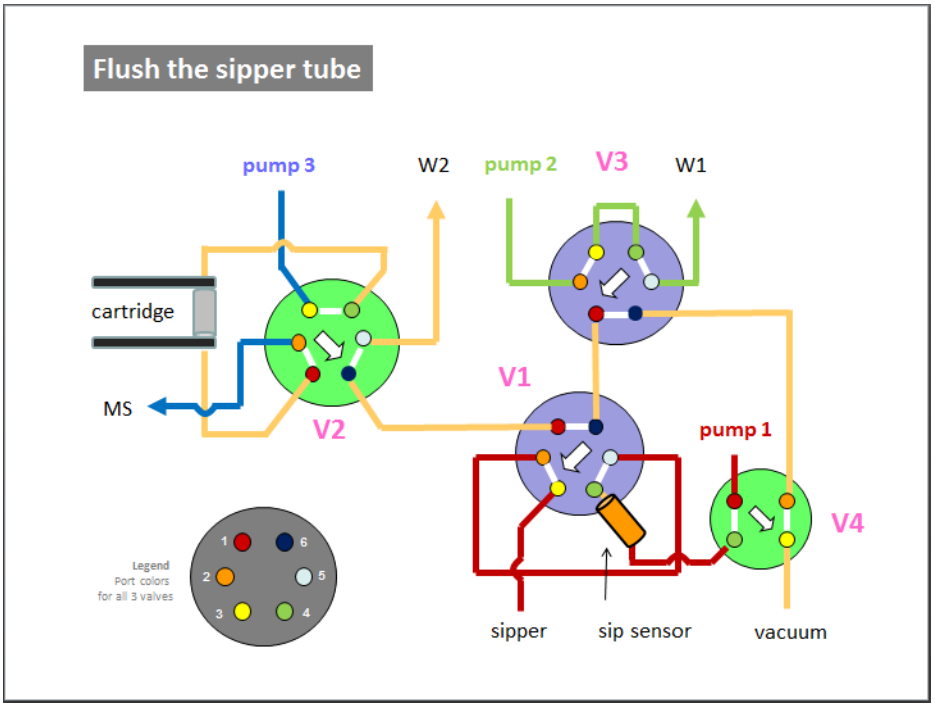
State 4: Elute flow path



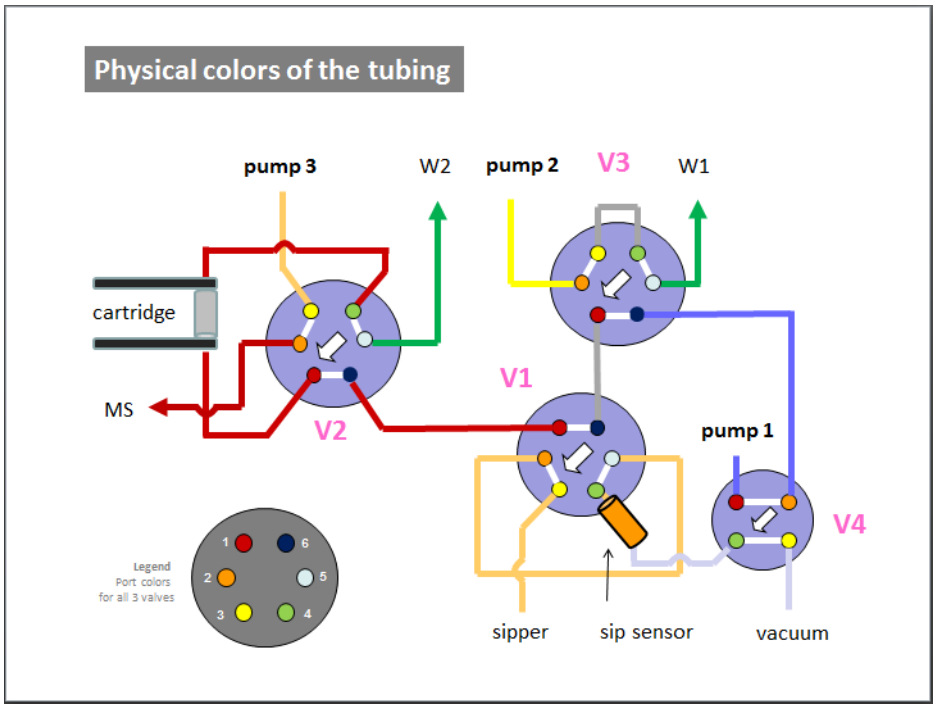
State 5: Re-equilibrate flow path



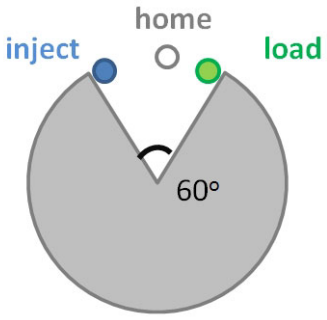
Flush the sipper flow path



Physical colors of the tubing



Valve positions



A Changing the sequence of the sipper operation

This section provides a description of the change in the default sip sequence operation in RapidFire Acquisition software version 6.1 and how to implement this change if you are running RapidFire Acquisition software version 6.0 or earlier. This section contains the following topics:

About the sip sequence operation 126
Changing the sequence of sip operation 127

About the sip sequence operation

In RapidFire Acquisition software 6.1, the sequence of sip operation was changed to decrease the timing variability between well sampling. As a consequence, this change also slightly increases the through-put of the system.

In RapidFire Acquisition software version 6.0 and earlier, the default sequence of sip operation is as follows:

1. Draw Sample 1
2. Move to Wash Stations
3. Wait for current injection to conclude
4. Move to next sample location
5. Draw Sample 2

In RapidFire Acquisition software 6.1, the default sequence of sip operation was changed to the following:

1. Draw Sample 1
2. Move to Wash Stations
3. Move to next sample location
4. Wait for current injection to conclude
5. Draw Sample 2

If you have RapdiFire Acquisition software 6.0, or earlier, you can change the default sequence of sip operation to the current (6.1 version) default. See the following procedure **“Changing the sequence of sip operation”** on page 127 for how to make this change.

Changing the sequence of sip operation

This procedure is for users upgrading to RapidFire Acquisition software 6.1 from version 6.0 or earlier.If this is a new RapidFire Acquisition software 6.1 installation, this change is included in the default cfg files.

Before you start

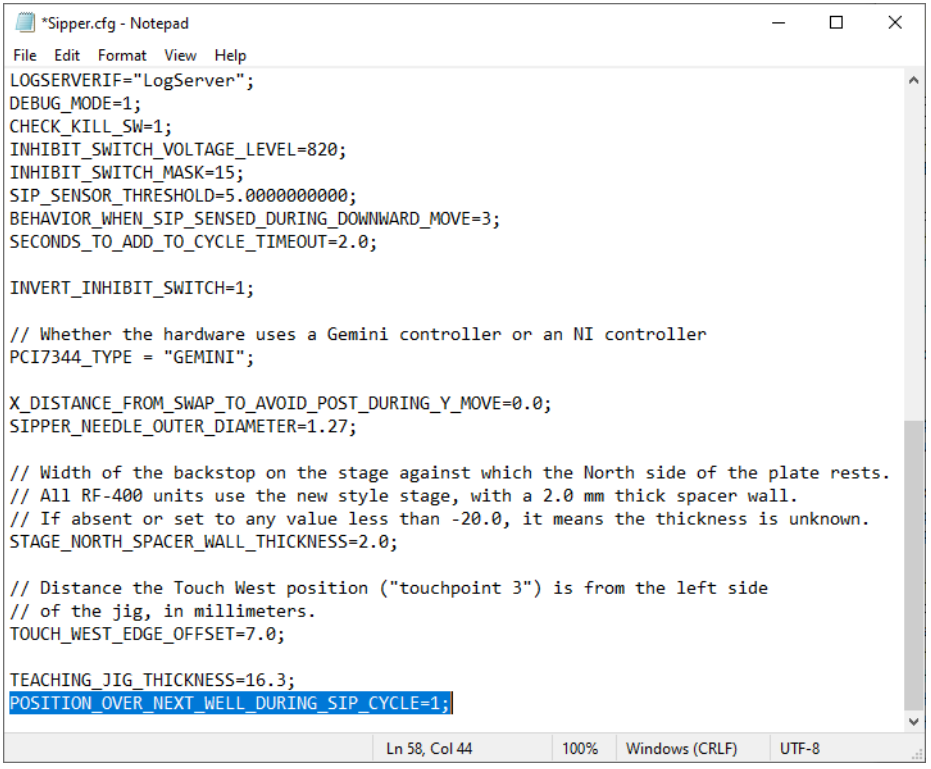
Close the RapidFire Control Panel and User Interface software, if running.

To change the sequence of sip operation:

1 Open the **Sipper.cfg** file in Notepad or other text editor. This file is located in C:\agilent\RapidFire\Cfgs(6.0.0) #SGxxxxxx (where #SGxxxxxx is the serial number of your system).

2 Scroll to the bottom of the file and add the following line of text:

POSITION_OVER_NEXT_WELL_DURING_SIP_CYCLE=1;



3 Save and close the file.

In This Book

This guide includes procedures for setup, operation, and maintenance of the RapidFire System.

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