

AriaMx SYBR Qualification Plate Kit

Part Number 5190-7708

Protocol

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

Version D2, March 2025



Agilent Technologies

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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.

In this Guide...

This protocol provides instructions for using the AriaMx SYBR Qualification Plate Kit.

1 Before You Begin

Make sure that you read and understand the information in this chapter before you start an experiment.

2 Protocol

This chapter provides instructions on running a SYBR Qualification test on the AriaMx Real-Time PCR System.

What's New in Version D2

- Added step for background calibration prior to running the qualification test. See [“Perform background calibration”](#) on page 10.

What's New in Version D1

- Removed *Note* from [page 8](#) about qualification of the AriaDx Real-Time PCR System.
- Updated terminology for optical modules on [page 8](#).

What's New in Version D0

- Updated contact information for Agilent Technical Support.

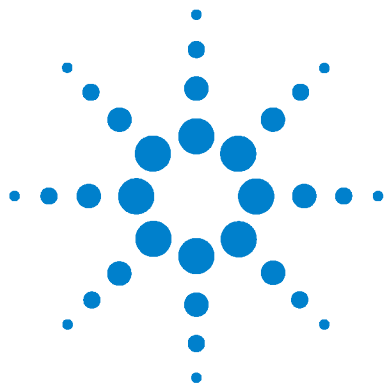
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Before You Begin

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Make sure that you read and understand the information in this chapter before you start an experiment.



Overview

The AriaMx SYBR Qualification Plate Kit is for use in a qualification test on the AriaMx Real-Time PCR System. Running a qualification test is one way to test for instrument errors. The wells of the AriaMx SYBR Qualification Plate are pre-filled with a QPCR master mix that includes the double-stranded DNA binding dye SYBR Green.

Instrument Optical Module Requirements

The AriaMx instrument must be equipped with a SYBR/FAM optical module and a ROX optical module to run a qualification test with the AriaMx SYBR Qualification Plate Kit.

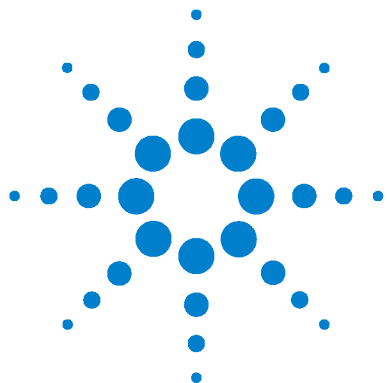
Kit components

The AriaMx SYBR Qualification Plate Kit contains one 96-well plate. Each of the 96 wells contains 20 μ L of QPCR reagents.

Plate storage and handling

The plate is shipped on dry ice. Store at -20°C upon receipt.

Wear chemical-resistant powder-free gloves whenever handling the SYBR Qualification Plate.



2 Protocol

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Run the test [10](#)

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This chapter provides instructions on running a SYBR Qualification test on the AriaMx Real-Time PCR System.



Perform background calibration

Prior to starting the qualification test, calibrate the background for the FAM and ROX optical modules. See the “Performing Background Calibration” section of the *AriaMx Real-Time PCR System Setup and User Guide* for instructions.

Prepare the plate

Prepare the plate just before starting the qualification test.

- 1** Thaw the AriaMx SYBR Qualification Plate at room temperature for 5–15 minutes. *Do not remove the seal.*
- 2** Once thawed, mix the contents of the wells by completing the following actions five times.
 - a** Invert the plate so that the liquid moves to the seal on top of each well.
 - b** Re-invert the plate to return the liquid to the bottom of each well.
- 3** After completing five mixing cycles, briefly spin the plate in a plate centrifuge at $500 \times g$ for at least one minute.
- 4** Verify that no bubbles are present at the bottoms of the wells, beneath the liquid.

While it is critical to remove any bubbles from the bottom of the tubes, small bubbles near the liquid meniscus should not affect the test results.

Run the test

Running a qualification test requires preparing the plate, running the experiment on the AriaMx instrument, and analyzing the results.

- 1** On your PC, open the AriaMx software.
- 2** At the top of the program window, click **Instrument > Qualification Test**.

A new tab opens for the Qualification Test experiment. The experiment opens to the Thermal Profile screen.

NOTE

The plate setup for the experiment is already populated with the necessary well properties and the thermal profile is already set to the correct parameters. You cannot edit the settings on the Plate Setup screen or the Thermal Profile screen.

3 Click Run.

The Instrument Explorer dialog box opens.

4 Locate the instrument that you will be running the SYBR Qualification test on and click Send Config.

A message box opens notifying you that you must save the experiment before you can connect to the instrument.

5 Click Save in the message box.

The Save As dialog box opens.

6 Select a folder for the experiment and type a name into the File name field (or use the default). Click Save.

7 Load the prepared SYBR Qualification Plate in the AriaMx thermal block then close the hot top lid and the instrument door.

8 On the instrument touchscreen, open the primed experiment and navigate to the Thermal Profile screen. Press Run Experiment.

The instrument starts running the qualification test experiment.

9 Return to the AriaMx software on your PC. The software directs you to the Raw Data Plots screen, where you can monitor the progress of the run.

10 At the end of the run, open the qualification test experiment in the AriaMx software on your PC.

11 Click Graphical Displays in the Experiment Area panel on the left side of the screen.

12 Check the panel on the right side of the screen to determine if the qualification test passed.

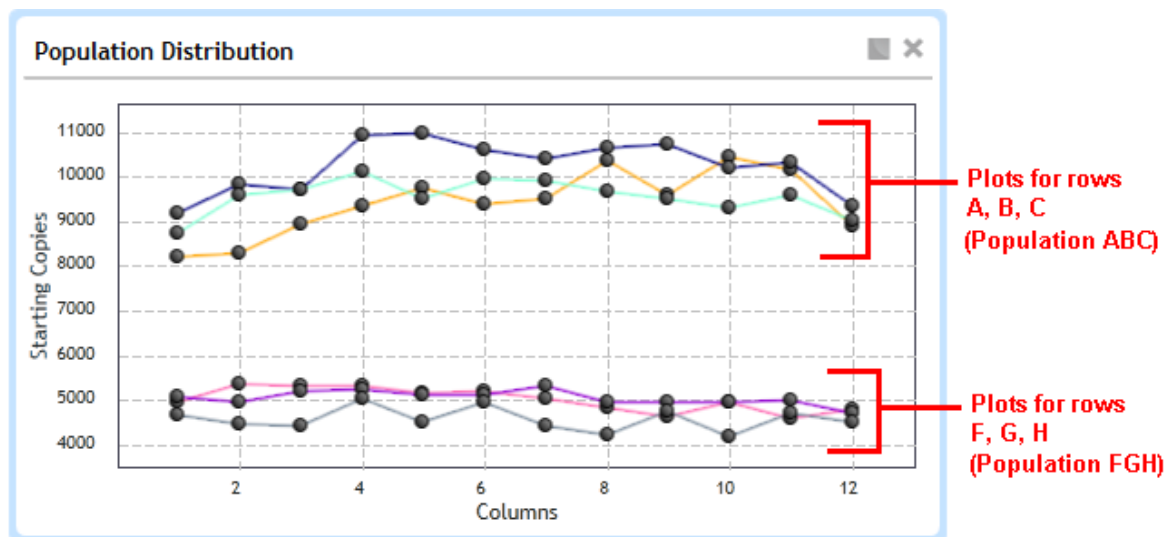
- If it passed, the panel displays **Results Pass**
- If it failed, the panel displays **Results Check**

If your qualification test failed, contact Agilent [Technical Support](#) for help with troubleshooting.

About the Qualification Test graphical data

For a qualification test, the Graphical Displays screen includes graphs for the Amplification Plots, the Standard Curve, and the Population Distribution.

The Population Distribution graph is unique to Qualification Test experiments. For each row of Unknown wells on the plate, this graph plots the number of initial template copies calculated for that row against the plate's column number. Because all wells in rows A, B, and C are replicates and all wells in rows F, G, and H are replicates, the distribution of the plot lines indicate the variability in initial template calculations across the thermal block within the two populations (the ABC population and the FGH population). Prior to amplification, the concentration of the template in the ABC population is twice the concentration of the template in the FGH population. In order to pass the qualification test, the AriaMx software requires that both populations be at least 98.5% distinct after outlier wells are culled. The percent distinct for the Population Distribution graph is displayed in the panel on the right side of the Graphical Displays screen (next to % **Distinct**). Note that outlier wells are plotted on the Population Distribution graph with a red x.



If your qualification test failed, Agilent's Technical Support staff may use the graphical data to help troubleshoot the cause of the failure.

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In This Book

This guide contains
information to run the
AriaMx SYBR
Qualification Plate Kit
protocol.

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