

Mx3000P/Mx3005P QPCR SYSTEM

INSTRUMENT QUALIFICATION β -ACTIN INSTALL PLATE INSTRUCTIONS

Plate Storage and Handling Instructions

The plate is shipped on dry ice. Store at -20°C upon receipt. Do not remove the foil wrapper until immediately before performing the run.

Wear chemical-resistant powder-free gloves whenever handling the qualification plate. This will ensure that no finger oils or other debris are transferred to the optical seal.

Preparing for the Qualification Test

Note *The Mx3000P or Mx3005P instrument and MxPro software must be set up, according to the instructions in the system Setup and User's Guide, prior to running the Qualification Test.*

Just before starting the Instrument Qualification Test run, thaw the plate at room temperature for 15–30 minutes, leaving the plate sealed in the foil wrapper while thawing.

After thawing, remove the foil wrapper and then mix the contents of the wells by completing the following actions **five times**:

- Invert the plate. Use a sharp, swift, downward motion to move the liquid to the seal on top of each well. Observe the liquid to verify that the liquid has moved from the bottoms to the tops of the tubes.
- Re-invert the plate, and use the same motion to return the liquid to the bottom of each well, again verifying liquid movement before proceeding.

After completing five mixing cycles, collect the liquid at the bottom of the wells. Brief centrifugation of the plate at 1000 rpm is highly recommended, in order to dislodge any bubbles and to collect the liquid.

If a plate centrifuge is not available, shake the liquid down to the bottom of the tubes (using the same motion described above for mixing). Shake-down the plate until the majority of the liquid is at the bottom of the tube and only minimal amounts of liquid are retained on the tube seal or sides. It is important to shake-down any noticeable liquid on the tube seal (e.g. visible semi-circles or crescent shapes). Small amounts of liquid spray retained on the tube sides should not affect the test results.

Prior to loading the plate, verify that no bubbles are present at the bottoms of the tubes, 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.

Important *Bubbles trapped at the bottom of the tubes will interfere with fluorescence readings, invalidating the test.*

Performing the Qualification Test Run

The first time the MxPro software is opened using an instrument-connected computer, the software provides a prompt to run the **Instrument Qualification Test**. The test may also be initiated by selecting **Qualification Test** from the **Instrument** menu. The software automatically loads the plate set-up and thermal profile for the qualification run.

A validation protocol Wizard will provide instructions in a series of dialog boxes before, during, and after the run. Since the Qualification Test is a fixed test, only the actions directed by the validation protocol Wizard can be completed. **Do not attempt to modify the Qualification Test experiment or to analyze data during the Qualification Test run.**

First, the **Instrument Qualification Test-Start** dialog opens. When the plate has been prepared for the run, click **Next** to open the **Instrument Qualification Test-Start Run** dialog box. Enter any comments for the qualification test. Verify that the Perfect Fit frame shipped with the instrument has been removed from the thermal block area. Load the test plate in the thermal block, verifying the correct orientation (well A1 in the top, left corner). Close the instrument door, and then select **Start Run**. The Mx3000P or Mx3005P instrument will begin the Instrument Qualification Test run. If the **Pre-Run Lamp Warm Up** dialog box appears, select **Run Now**.

Important *While the Qualification Test experiment is running, do not open the instrument door or cancel the run.*

Data Analysis

When run is complete, the software will proceed to the Analysis-Text Report section and the **Instrument Qualification Test-Run Complete** dialog box will appear. Select **Analyze**. The software will open the Microsoft Excel Instrument Qualification Validation template and export the Text Report data into the template.

Notes *If a **Server Busy** error dialog box appears, click **Retry** until the data export succeeds.*

*If any Norton anti-virus dialog boxes appear, select **Notify again in 1 Day**, and close the dialog.*

Qualification Validation

The Microsoft Excel Validation template will open to the **FAM analysis** tab. Verify that the **Data Count** cell reports the result **Good** to ensure that all of the data has been correctly exported into Excel. Next, verify that the **Result** cell reports a **Pass** result. A **Pass** result indicates that the instrument is working properly. In this case, it is not necessary to E-mail the data to Stratagene Technical Services.

If the test reports a **Check** result, it is important to call Stratagene Technical Services and send the three data files, including Customer Information.txt, into Stratagene Technical Services by E-mail for troubleshooting guidance. Include the name of your institution, a contact name, and the instrument serial number when sending the data to Technical Services.

Instrument Qualification Completion

After checking the Microsoft Excel Validation template, go back into the MxPro software. For a test report **Check** result only, the **Instrument Qualification Test-E-mail Experiment** dialog box will appear and present the option to automatically **E-mail** the data or save the data to a disk to later E-mail the data to Stratagene Technical Services (QPCR@agilent.com). Finally, the **Instrument Qualification Test-Complete** dialog box will appear, indicating that the test is finished.

Stratagene Technical Services

USA/Canada (Toll-free): 800 894 1304, x2

Japan: 3-5821-8076

Other countries: Visit www.genomics.agilent.com; under Support click Contact Us

Email techservices@agilent.com

World Wide Web www.genomics.agilent.com