



Technical Bulletin

Technical Information for Quidel Molecular RSV + hMPV Assay on Qiagen's Rotor-Gene Q

Quidel Corporation has verified the performance of the Quidel Molecular RSV + hMPV Assay on Qiagen's Rotor-Gene Q, software version 2.0.2.4. Internal studies performed by Quidel have demonstrated performance on this instrument is substantially equivalent to analytical and clinical performance data found in the package insert.

Due to variations in thermocyclers there are different settings and additional programming procedures that should be followed for the Rotor-Gene Q software 2.0.2.4. These procedures are detailed on pages 2-6.

Please call Quidel Technical Support at 858.552.1100 or technicalsupport@quidel.com if you have any questions regarding the Quidel Molecular RSV + hMPV Assay or any other Quidel product. Our hours of operation are Monday through Friday, between 8:00 a.m. to 5:00 p.m., Eastern Time.

You may also visit our website at quidel.com for information on any of Quidel's product lines. Other product information available on our website includes: CPT codes, CLSI procedure guidelines, MSDS, and package inserts.



RSV + hMPV Assay

Supplemental Instructions: Creating a Rotor-Gene Q, software version 2.0.2.4, RSV + hMPV Assay Protocol Template

Purpose: The following supplemental instructions will aid in programming an assay template for Rotor-Gene Q software version 2.0.2.4, from Qiagen to run the Quidel Molecular RSV + hMPV RT-PCR assay kit. Seek specific training or guidance if you are unfamiliar with the use of this platform. For assistance with this protocol, please contact Quidel Technical Support directly.

Limitations: The following protocol was developed for use with Quidel Molecular RSV + hMPV RT-PCR kit specifically. Its suitability for other assays is unknown. Check with Qiagen to ensure software compatibility.

Programming Instructions:

1. Launch the Rotor-Gene Q software package
2. In the **New Run** pop-up window select the **Advanced** tab on the top of the screen
3. Select **Empty Run** and then **New** on the lower right of the pop-up window to start the **Advanced Run Wizard**
 - a. Select the appropriate rotor size in the **Advanced Run Wizard** on the upper left of the screen
 - b. Check the box that states the **Locking Ring is Attached** and select **Next**
 - c. Leave the **Operator** and **Notes** sections empty
 - d. Enter **20 µl** as the **Reaction Volume** in the lower left of the screen
 - e. For the **Sample Layout** choose **1, 2, 3...** and then select **Next**
 - f. Under **Channel Setup** select **Create New** to enter information for each detector
 - i. Under **Name** enter **RSV**
 - ii. **Source** select 470 nm
 - iii. **Detector** select 510 nm
 - iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
 - v. Select **OK**
 - g. Repeat the step above by selecting **Create New**
 - i. Under **Name** enter **hMPV**
 - ii. **Source** select 585 nm
 - iii. **Detector** select 610 nm
 - iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
 - v. Select **OK**
 - h. Repeat the step above by selecting **Create New**
 - i. Under **Name** enter **PRC**
 - ii. **Source** select 625 nm
 - iii. **Detector** select 660 nm
 - iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
 - v. Select **OK**
 - i. Select the **Edit Profile** button in the middle of the window to setup a cycling profile
 - i. In the **Edit Profile** window go to the upper left of the screen to **New** and in the drop-down menu select **Cycling**. A hold and three step cycling stage should appear.
 - ii. Modify the hold stage to have a temperature at **55°C** and a time of **5:00 minutes**

- iii. Select the **Insert After** button in the middle of the pop-up window and then select **New Hold at Temperature**
- iv. Modify the second hold stage to have a temperature at **60°C** and a time of **5:00 minutes**
- v. Select the **Insert After** button in the middle of the pop-up window and then select **New Hold at Temperature** to insert a third hold stage
- vi. Modify the third hold stage to have a temperature at **65°C** and a time of **5:00 minutes**
- vii. Highlight the first **cycling stage** and modify it as follows:
 1. This cycle repeats **10** time(s)
 2. Select **Timed Step** from the drop-down menu in the middle left of the screen
 3. Do not select **Long Range** or **Touchdown** on the left of the screen
 4. The first step:
 - a. **92°C**
 - b. **5 seconds**
 - c. **Not Acquiring**
 5. Select step two and set as follows:
 - a. **57°C**
 - b. **40 seconds**
 - c. **Not Acquiring**
 6. Highlight step three and delete it by selecting the “-” button in the middle of the window
 7. Select the **Insert After** button in the middle of the pop-up window and then select **New Cycling**
- viii. Highlight the second **cycling stage** and modify it as follows:
 1. This cycle repeats **35** time(s)
 2. Select **Timed Step** from the drop-down menu in the middle left of the screen
 3. Do not select **Long Range** or **Touchdown** on the left of the screen
 4. The first step:
 - a. **92°C**
 - b. **5 seconds**
 - c. **Not Acquiring**
 5. Select step two and set as follows:
 - a. **57°C**
 - b. **40 seconds**
 - c. Select **Acquiring to Cycling A**
 - i. **Under Acquiring Channels** highlight the default channel name (Green) and select the < button to move it over to the **Available Channels** list
 - ii. In the **Available Channels** list select **RSV** and select the > button to move it over to the **Acquiring Channels** list
 - iii. Repeat the step above for **hMPV** and the **PRC** and then select **OK**
 6. Highlight step three and delete it by selecting the “-” button in the middle of the window
- ix. In the **Edit Profile** window select **OK**
- j. In the **New Run Wizard** window select **Gain Optimisation**
 - i. In the middle of the **Auto-Gain Optimisation Setup** window select the drop-down menu under **Channel Settings** and select **RSV**.
 - ii. Select the **Add** button on the right
 1. In the **Auto-Gain Optimization Channel Settings** window ensure that the **RSV Tube Position** is set to **1**. This requires that a positive control, containing RSV, hMPV, and PRC, be tested with each PCR run and placed in the first tube. Failure to do so may cause the gain to be incorrectly set.

2. Leave the **Target Sample Range** and the **Acceptable Gain Range** set to the defaults, 5-10FI and –10 to 10 respectively.
3. Select **OK**
4. Repeat steps 3. j. ii. 1-3. for **hMPV** and the **PRC**
- iii. In the **Auto-Gain Optimisation Setup** window check the box next to **Perform Optimisation Before 1st Acquisition**
- iv. Select **Close**
- k. In the **New Run Wizard** window select the **Next** button
- l. Save the new protocol as a template for future use
 - i. On the bottom right of the window select the **Save Template** button
 - ii. **Save In:** C:\Program Files\Rotor-Gene Q Software\Templates
 - iii. **File name:** 'Quidel Molecular RSV+hMPV'
 - iv. **Save as type:** 'Template (*.ret)'
- m. Exit the software



RSV + hMPV Assay

Supplemental Instructions: Analyzing a Rotor-Gene Q, software version 2.0.2.4, RSV+hMPV Assay Run

Purpose: The following supplemental instructions will aid in analyzing a Quidel Molecular RSV + hMPV RT-PCR assay run on the Rotor-Gene Q, software version 2.0.2.4, from Qiagen. Seek specific training or guidance if you are unfamiliar with the use of this platform. For assistance with this analysis, please contact Quidel Technical Support directly.

Limitations: The following analysis was developed for use with Quidel Molecular RSV + hMPV RT-PCR kit specifically. Its suitability for other assays is unknown. Check with Qiagen to ensure software compatibility.

Analysis Instructions:

1. Open the run file that needs to be analyzed
2. In the upper menu toolbar select the **Analysis** button
 - a. Select **Quantitation**, then **Cycling A. RSV**, and **Show**
 - b. The threshold needs to be set for **RSV**
 - i. In the far right bottom of the screen under **CT Calculation** enter **0.025** for the **RSV Threshold**
 - ii. In the **Eliminate Cycles before** box ensure the default of **1** is entered
 - iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the graph states Linear Scale or Log Scale)
 - iv. In the tool bar at the top of the amplification graph select the following:
 1. Dynamic Tube
 2. Slope Correct
 3. Ignore first
 - a. In the pop-up menu, enter **5** and select **OK**
 4. Outlier Removal
 - a. In the pop-up menu, under **NTC Threshold**, enter **10**
 - b. Ensure that the Enabled box under **Reaction Efficiency Threshold** is **NOT** selected
 - c. Select **OK**
 - c. Select **Quantitation**, then **Cycling A. hMPV**, and **Show**
 - d. The threshold needs to be set for **hMPV**
 - i. In the far right bottom of the screen under **CT Calculation** enter **0.06** for the **hMPV Threshold**
 - ii. In the **Eliminate Cycles before** box ensure the default of **1** is entered
 - iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the graph states Linear Scale or Log Scale)
 - iv. In the tool bar at the top of the amplification graph select the following:
 1. Dynamic Tube
 2. Outlier Removal
 - a. In the pop-up menu, under **NTC Threshold**, enter **5**
 - b. Ensure that the Enabled box under **Reaction Efficiency Threshold** is **NOT** selected
 - c. Select **OK**

- e. Select **Quantitation**, then **Cycling A. PRC**, and **Show**
- f. The threshold needs to be set for PRC
 - i. In the far right bottom of the screen under **CT Calculation** enter **0.075** for the **PRC Threshold**
 - ii. In the **Eliminate Cycles before** box ensure the default of **1** is entered
 - iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the graph states Linear Scale or Log Scale)
 - iv. In the tool bar at the top of the amplification graph select the following:
 1. Dynamic Tube
 2. Slope Correct
 3. Ignore first
 - a. In the pop-up menu, enter **5** and select **OK**
 4. Outlier Removal
 - a. In the pop-up menu, under **NTC Threshold**, enter **10**
 - b. Ensure that the Enabled box under **Reaction Efficiency Threshold** is **NOT** selected
 - c. Select **OK**

Interpretation of the Quidel Molecular RSV+hMPV Assay Results on the Rotor-Gene Q				
Assay Result	Detector: RSV	Detector: hMPV	Detector: Process Control	Interpretation of Results
Negative	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	5.0 ≤ Ct ≤ 35.0	No RSV or hMPV viral RNA detected; PRC detected
RSV Positive	5.0 ≤ Ct ≤ 35.0	Ct < 5.0 or Ct >35.0	NA*	RSV viral RNA detected
hMPV Positive	Ct < 5.0 or Ct > 35.0	5.0 ≤ Ct ≤ 35.0	NA*	hMPV viral RNA detected
RSV and hMPV Positive	5.0 ≤ Ct ≤ 35.0	5.0 ≤ Ct ≤ 35.0	NA*	RSV and hMPV viral RNA detected
Invalid	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	No RSV or hMPV viral RNA and no PRC detected; invalid test. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same sample or obtain a new sample and retest.

*No Ct value is required for the Process Control to make a positive call.

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