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

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. 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

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, 5-10FV 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

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**Interpretation of the Quidel Molecular RSV+hMPV Assay Results on the Rotor-Gene Q**

<table>
<thead>
<tr>
<th>Assay Result</th>
<th>Detector: RSV</th>
<th>Detector: hMPV</th>
<th>Detector: Process Control</th>
<th>Interpretation of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Ct &lt; 5.0 or Ct &gt; 35.0</td>
<td>Ct &lt; 5.0 or Ct &gt; 35.0</td>
<td>5.0 ≤ Ct ≤ 35.0</td>
<td>No RSV or hMPV viral RNA detected; PRC detected</td>
</tr>
<tr>
<td>RSV Positive</td>
<td>5.0 ≤ Ct ≤ 35.0</td>
<td>Ct &lt; 5.0 or Ct &gt; 35.0</td>
<td>NA*</td>
<td>RSV viral RNA detected</td>
</tr>
<tr>
<td>hMPV Positive</td>
<td>Ct &lt; 5.0 or Ct &gt; 35.0</td>
<td>5.0 ≤ Ct ≤ 35.0</td>
<td>NA*</td>
<td>hMPV viral RNA detected</td>
</tr>
<tr>
<td>RSV and hMPV Positive</td>
<td>5.0 ≤ Ct ≤ 35.0</td>
<td>5.0 ≤ Ct ≤ 35.0</td>
<td>NA*</td>
<td>RSV and hMPV viral RNA detected</td>
</tr>
<tr>
<td>Invalid</td>
<td>Ct &lt; 5.0 or Ct &gt; 35.0</td>
<td>Ct &lt; 5.0 or Ct &gt; 35.0</td>
<td>Ct &lt; 5.0 or Ct &gt; 35.0</td>
<td>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.</td>
</tr>
</tbody>
</table>

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