



Technical Bulletin

Technical Information for Quidel Molecular Influenza A+B Assay on Roche's LightCycler® 480 Instrument II

Quidel Corporation has verified the performance of the Quidel Molecular Influenza A+B Assay on Roche's LightCycler® 480 Instrument II (software version 1.5.0.39). Internal studies performed by Quidel have demonstrated that the performance on this instrument is substantially equivalent to analytical and clinical performance data found in the package insert.

The cycling conditions for the LightCycler® 480 Instrument II are identical; however due to variation in thermocyclers there are different settings, additional programming procedures, and an interpretation table that should be followed for the LightCycler® 480 Instrument II. These procedures are detailed on pages 2-5.

For technical support on the Quidel Molecular Influenza A+B Assay, please call 1+ 800-874-1517 or (858) 552-1100 (outside the U.S.), Monday through Friday, between 8:00 a.m. and 5:00 p.m., Eastern Time.

For e-mail support contact technicalsupport@quidel.com

You may also visit our website at quidel.com for this or any other Quidel product.

Influenza A+B Assay

Supplemental Instructions: Creating a LC 480 II Assay Run Template

Purpose: The following supplemental instructions will aid in programming a run template for the LightCycler[®] 480 Instrument II from Roche to run the Quidel Molecular Influenza A+B 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 Influenza A+B RT-PCR kit specifically. Its suitability for other assays is unknown. Check with Roche to ensure software compatibility.

Programming Instructions:

1. Launch the LightCycler (LC) 480 software package
2. The **Detection Format** must be established to specify the channels in which fluorescence will be read
 - a. Select **Tools** in the startup screen in the lower right of the screen
 - b. Select **Detection Formats** then choose **New**
 - c. Name the format Quidel Molecular Influenza A+B
 - d. In the **Filter Combination Selection** window select 465-510, 533-580 and 618-660
 - e. In the **Selected Filter Combination List** window under name type in Influenza A for 465-510, Influenza B for 533-580 and PRC for 618-660
 - f. Leave all default setting values to 1 under Melt Factor, Quant Factor, and Max Integration Time
 - g. Select **Close** to save the new detection format and return to startup screen
 - h. To access this newly created **Detection Format**, the LC 480 software must be closed, then reloaded
3. After closing and reloading the software select **White Plates** and **New Experiment** under Experiment Creation window
4. On the next screen select "Quidel Molecular Influenza A+B" from the pull-down menu under **Detection Formats**
5. Enter **20ul** as the **Reaction Volume** in the upper right of the screen
6. Enter the names for each of the RT-PCR programs
 - a. Under **Program Name** enter **Stage 1**, under **Cycles** enter **1**, and in **Analysis Mode** select **none**
 - b. Select the "+" icon to add a program
 - c. Name the next program **Stage 2**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
 - d. Select the "+" icon to add a program
 - e. Name the next program **Stage 3**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
 - f. Select the "+" icon to add a program
 - g. Name the next program **Stage 4**, under **Cycles** enter **50**, and in the **Analysis Mode** select **quantification**
7. Set the RT-PCR cycling times and temperatures
 - a. Highlight **Stage 1** under **Program Name** and change **Stage 1 Temperature Targets** as follows:
 - i. **Target (°C)** set to **55**
 - ii. **Acquisition Mode** select **none**
 - iii. **Hold (hh:mm:ss)** set to **5:00**
 - iv. **Ramp Rate (°C/s)** to **4.4**
 - v. **Sec Target (°C), Step Size (°C), and Step Delay (cycles)** will be left at 0 for stages 1-4.

- b. Highlight **Stage 2** under **Program Name** and change **Stage 2 Temperature Targets** as follows:
 - i. **Target (°C)** set to **60**
 - ii. **Acquisition Mode** select **none**
 - iii. **Hold (hh:mm:ss)** set to **5:00**
 - iv. **Ramp Rate (°C/s)** to 4.4
- c. Highlight **Stage 3** under **Program Name** and change **Stage 3 Temperature Targets** as follows:
 - i. **Target (°C)** set to **65**
 - ii. **Acquisition Mode** select **none**
 - iii. **Hold (hh:mm:ss)** set to **5:00**
 - iv. **Ramp Rate (°C/s)** to 4.4
- d. Highlight **Stage 4** under **Program Name** and change **Stage 4 Temperature Targets** as follows:
 - i. The first step:
 1. **Target (°C)** set to **92**
 2. **Acquisition Mode** select **none**
 3. **Hold (hh:mm:ss)** set to **0:05**
 4. **Ramp Rate (°C/s)** to 4.4
 - ii. Select the “+” icon to add a step and set the second step:
 1. **Target (°C)** set to **57**
 2. **Acquisition Mode** select **single**
 3. **Hold (hh:mm:ss)** set to **0:40**
 4. **Ramp Rate (°C/s)** to 2.2
8. Save the new protocol as a run template for future use.
 - a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
 - b. Choose **Save As Template**
 - c. Select the **Templates Folder**
 - d. Highlight **Run Templates Folder**
 - e. Name the template ‘Quidel Molecular Influenza A+B run template’ and click the “check” button
9. Exit the software.

Influenza A+B Assay

Supplemental Instructions: Creating a LC 480 II Assay Analysis Template

Purpose: The following supplemental instructions will aid in programming an analysis template for the LightCycler[®] 480 Instrument II from Roche to run the Quidel Molecular Influenza A+B 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 Influenza A+B RT-PCR kit specifically. Its suitability for other assays is unknown. Check with Roche to ensure software compatibility.

Programming Instructions:

1. The analysis template can only be established after the initial experiment has completed
2. On the Quidel Molecular Influenza A+B run select the **Analysis** button in the module bar
 - a. Choose **Abs Quant/Fit Points**
 - b. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop down menu and then select the “check” button
 - c. Set the **Background** to 2-10 for all analytes
 - i. Set **Min Offset** to 1
 - ii. Set **Max Offset** to 9
 - d. In the center bottom of the screen ensure that **Color Compensation** is off for all analytes
 - e. Leave the default settings as **First Cycle 1** and **Last Cycle 50**
3. At the top middle of the screen select **Noise Band**
4. Choose the pull-down menu next to the **Noise Band** button and select **Noise Band Fluorescence**
5. For each analyte under the **Filter Comb** button, set the noise band as follows:
 - a. Influenza A set to 1.95
 - b. Influenza B set to 1.2
 - c. PRC set to 1.4619
6. Choose **Calculate** in the bottom left of the screen
7. Save the new analysis protocol as a template for future use
 - a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
 - b. Choose **Save As Template**
 - c. Select the **Templates Folder**
 - d. Highlight **Analysis Templates Folder**
 - e. Name the template Quidel Molecular Influenza A+B analysis template and click the “check” button
8. Create a report
 - a. Select the **Save** icon on the global action bar on the right side of the screen
 - b. Choose the **Report** button on the module bar on the left of the screen
 - c. Select the appropriate settings and press the **Generate** button
9. To apply an Analysis Template to subsequent runs
 - a. Once the run has finished select the **Analysis** button in the module bar
 - b. Choose **Abs Quant/Fit Points**
 - c. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop down menu and then select the “check” button

- d. Select the **Apply Template** button on the far left of the screen and choose the Quidel Molecular Influenza A+B analysis template from the **Analysis Templates Folder**
- e. Select yes in the pop-up window

10. Interpretation of results

Interpretation of the Quidel Molecular Influenza A+B Assay Results on the LC 480 Thermocycler				
Assay Result	Detector: Influenza A	Detector: Influenza B	Detector: Process Control	Interpretation of Results
Negative	CP < 10.0 or CP > 50.0	CP < 10.0 or CP > 50.0	10.0 ≤ CP ≤ 50.0	No Influenza A or Influenza B viral RNA detected; PRC Detected
Influenza A Positive	10.0 ≤ CP ≤ 50.0	CP < 10.0 or CP > 50.0	NA*	Influenza A viral RNA detected
Influenza B Positive	CP < 10.0 or CP > 50.0	10.0 ≤ CP ≤ 50.0	NA*	Influenza B viral RNA detected
Influenza A and B Positive	10.0 ≤ CP ≤ 50.0	10.0 ≤ CP ≤ 50.0	NA*	Influenza A and Influenza B viral RNA detected
Invalid	CP < 10.0 or CP > 50.0	CP < 10.0 or CP > 50.0	CP < 10.0 or CP > 50.0	No Influenza A or Influenza B 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 CP value is required for the Process Control to make a positive call.

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