



Technical Bulletin

Technical Information for Quidel Molecular Direct *C. difficile* Assay on Roche's LightCycler® 480 Instrument II

Quidel Corporation has verified the performance of the Quidel Molecular Direct *C. difficile* Assay on Roche's LightCycler® 480 Instrument II, software version 1.5.0.39. 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 differences in instrument platforms, new programming procedures for the LightCycler 480 Instrument II software 1.5.0.39 are provided. These procedures are detailed on pages 2-4.

For technical support on the Quidel Molecular Direct *C. difficile* 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.

C. difficile Assay

Supplemental Instructions: Creating a LC 480 II *C. difficile* 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 Direct C. difficile 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 Direct C. difficile 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 C. difficile"
 - d. In the **Filter Combination Selection** window select 533-580 and 618-660
 - e. In the **Selected Filter Combination List** window under name type in *C. difficile* 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 C. difficile" 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 **15**, 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 **35**, 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 **92**
 - ii. **Acquisition Mode** select **none**
 - iii. **Hold (hh:mm:ss)** set to **2:00**
 - iv. **Ramp Rate (°C/s)** to **3.5**
 - v. **Sec Target (°C), Step Size (°C), and Step Delay (cycles)** will be left at 0 for stages 1-3.

- b. Highlight **Stage 2** under **Program Name** and change **Stage 2 Temperature Targets** as follows:
 - i. The first step:
 1. **Target (°C)** set to **88**
 2. **Acquisition Mode** select **none**
 3. **Hold (hh:mm:ss)** set to **0:05**
 4. **Ramp Rate (°C/s)** to 2.2
 - ii. Select the “+” icon to add a step and set the second step:
 1. **Target (°C)** set to **57**
 2. **Acquisition Mode** select **none**
 3. **Hold (hh:mm:ss)** set to **0:05**
 4. **Ramp Rate (°C/s)** to 2.2
 - iii. Select the “+” icon to add a step and set the third step:
 1. **Target (°C)** set to **63**
 2. **Acquisition Mode** select **none**
 3. **Hold (hh:mm:ss)** set to **0:35**
 4. **Ramp Rate (°C/s)** to 3.5
- c. Highlight **Stage 3** under **Program Name** and change **Stage 3 Temperature Targets** as follows:
 - i. The first step:
 1. **Target (°C)** set to **88**
 2. **Acquisition Mode** select **none**
 3. **Hold (hh:mm:ss)** set to **0:05**
 4. **Ramp Rate (°C/s)** to 2.2
 - ii. Select the “+” icon to add a step and set the second step:
 1. **Target (°C)** set to **57**
 2. **Acquisition Mode** select **none**
 3. **Hold (hh:mm:ss)** set to **0:05**
 4. **Ramp Rate (°C/s)** to 2.2
 - iii. Select the “+” icon to add a step and set the third step:
 1. **Target (°C)** set to **63**
 2. **Acquisition Mode** select **single**
 3. **Hold (hh:mm:ss)** set to **0:35**
 4. **Ramp Rate (°C/s)** to 3.5
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 Direct C. difficile” run template and click the “check” button
9. Exit the software.



C. difficile Assay

Supplemental Instructions: Creating a LC 480 II C. difficile 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 Direct C. difficile 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 Direct C. difficile RT-PCR kit specifically. Its suitability for other assays is unknown. Check with Roche to ensure software compatibility.

Analysis Instructions:

1. On the Quidel Molecular C. difficile run select the **Analysis** button in the module bar
 - a. Choose **Abs Quant/2nd Derivative Max**
 - b. In the **create new analysis** pop-up window select the drop down menu next to **subset** and select your pre-defined subset
 - c. Select the “check” button in the pop-up window
 - d. In the center bottom of the screen ensure that **Color Compensation** is off for all analytes
 - e. Select *C. difficile* or PRC by choosing the appropriate wavelength under the **Filter Comb** button
 - f. Leave the default settings for **Use Efficiency, Mean, and High Confidence** buttons
2. Choose **Calculate** in the bottom left of the screen
3. Create a report for each filter combination.
 - 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
4. Interpreting Results
 - a. Review Cp values for each filter combination.
 - b. Any specimen which has no Cp value reported in the *C. difficile* filter combination must have a confirmed Cp value in the PRC filter combination before it can be reported as “Negative”.

| Interpretation of the Quidel Molecular Direct C. difficile Assay Results on LightCycler 480 | | | |
|---|----------------------------------|------------------------------|--|
| Assay Result | Detector: <i>C. difficile</i> | Detector: Process Control | Interpretation of Results |
| Negative | 5.0 > Cp > 35.0 | 5.0 ≤ Cp ≤ 35.0 | No <i>C. difficile</i> DNA detected |
| <i>C. difficile</i> Positive | 5.0 ≤ Cp ≤ 35.0 | NA* | <i>C. difficile</i> DNA detected. |
| Invalid | 5.0 > Cp > 35.0 | 5.0 > Cp > 35.0 | No <i>C. difficile</i> DNA and No PRC detected; invalid test, Retest the same purified sample. If the test is also invalid, re-test another aliquot of the same sample or obtain a new sample and re-test. |

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

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