

A stylized, multi-colored DNA double helix graphic that spans the width of the page, with colors transitioning from purple to yellow to blue. The text "Technical Bulletin" is overlaid on this graphic.

Technical Bulletin

Technical Information for Quidel Molecular Direct *C. difficile* Assay on Qiagen's Rotor-Gene Q

Quidel Corporation has verified the performance of the Quidel Molecular Direct *C. difficile* 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 2-5.

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

Supplemental Instructions: Creating a Rotor-Gene Q C. difficile Assay Protocol Template

Purpose: The following supplemental instructions will aid in programming an assay template for Rotor-Gene Q from Qiagen to run the Quidel Molecular Direct C. difficile 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 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 **72-Well 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 **20ul** 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 **C. difficile**
 - ii. **Source** select 530nm
 - iii. **Detector** select 555nm
 - 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 **PRC**
 - ii. **Source** select 625nm
 - iii. **Detector** select 660nm
 - iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
 - v. Select **OK**
 - h. 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 **92°C** and a time of **2:00 minutes**
 - iii. Highlight the first **cycling stage** and modify it as follows:
 1. In the small box in the middle of the screen select this cycle to repeat **15** 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. **5 seconds**
 - c. **Not Acquiring**
 - 6. Select step three and set as follows:
 - a. **68°C**
 - b. **25 seconds**
 - c. Select the **Acquiring to Cycling A** button on the left of the window
 - d. In the **Acquisition** pop-up window select **Don't Acquire**
 - 7. Select the **Insert After** button in the middle of the pop-up window and then select **New Cycling**
 - iv. 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. **94°C**
 - b. **5 seconds**
 - c. **Not Acquiring**
 - 5. Select step two and set as follows:
 - a. **57°C**
 - b. **5 seconds**
 - c. **Not Acquiring**
 - 6. Select step three and set as follows:
 - a. **63°C**
 - b. **35 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 **C. difficile** and select the > button to move it over to the **Acquiring Channels** list
 - iii. Repeat the step above for the **PRC** and then select **OK**
 - v. In the **Edit Profile** window select **OK**
 - i. 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 C. difficile.
 - ii. Select the **Add** button on the right
 - 1. In the **Auto-Gain Optimisation Channel Settings** window ensure that the C. difficile **Tube Position** is set to **1**. This requires that a positive control, containing C. difficile 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. i. ii. 1-3. for the **PRC**
- iii. In the **Auto-Gain Optimisation Setup** window check the box next to **Perform Optimisation Before 1st Acquisition**
- iv. Select **Close**
- j. In the **New Run Wizard** window select the **Next** button
- k. 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 C. difficile'
 - iv. **Save as type:** 'Template (*.ret)'
- l. Exit the software

Supplemental Instructions: Analyzing a Rotor-Gene Q C. difficile Assay Run

Purpose: The following supplemental instructions will aid in analyzing a Quidel Molecular Direct C. difficile assay run on the Rotor-Gene Q 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 Direct C. difficile 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 sample edit page under the **Selected** column select **No** for those tubes that have no samples
3. In the upper menu toolbar select the **Analysis** button
 - a. Select **Quantitation**, then **Cycling A. C. difficile**, and **Show**
 - b. The threshold needs to be set for C. difficile
 - i. In the far right bottom of the screen under **CT Calculation** enter **0.017** for the **C. difficile 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). Leave the button so that it says **Linear Scale** will then be in log scale).
 - iv. Do not change the other **Scale** options for the graph (Adjust Scale, Auto-Scale, or Default Scale).
 - c. Select **Quantitation**, then **Cycling A. PRC**, and **Show**
 - d. The threshold needs to be set for PRC
 - i. In the far right bottom of the screen under **CT Calculation** enter **0.025** 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). Leave the button so that it says **Linear Scale** will then be in log scale).
4. Save the analysis by pressing the **Save** button on the upper left of the screen
5. Exit the software

Interpretation of the Quidel Molecular Direct C. difficile Assay Results on Rotor-Gene Q			
Assay Result	Detector: C. difficile	Detector: Process Control	Interpretation of Results
Negative	$5.0 > Ct > 35.0$	$5.0 \leq Ct \leq 35.0$	No C. difficile DNA detected
C. difficile Positive	$5.0 \leq Ct \leq 35.0$	N/A	C. difficile DNA detected. Detection of PRC is not required when C. difficile is present.
Invalid	$5.0 > Ct > 35.0$	Fail	No C. difficile DNA and No PRC detected; invalid test, re-test 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.

0707ID0213I (02/13)