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Technical Bulletin

Technical Information for Quidel Molecular Direct C. difficile Assay on Bio-Rad's CFX96 Touch

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Quidel Corporation has verified the performance of the Quidel Molecular Direct C. difficile Assay on Bio-Rad's CFX96 Touch, software version 3.0. 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 CFX96 Touch software 3.0. 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 CFX96 Touch C. difficile Assay Protocol Template

Purpose: The following supplemental instructions will aid in programming an assay template for CFX96 Touch from Bio-Rad 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 Bio-Rad to ensure software compatibility.

Programming Instructions:

1. Launch the CFX96 Touch software package
2. In the **Startup Wizard** pop-up window **Select instrument** to be **CFX96** from the drop down menu
3. Under **Select Run Type** press the **User-defined** button
4. Create a new thermocycler protocol by selecting **Create New** from the **Run Setup** window
5. Make the following changes to the cycling conditions in the **Protocol Editor:**
    a. Change the **Sample Volume** to **20ul**
    b. Under **Tools** in the top left toolbar select **Run Time Calculator** and check **96 Wells-All Channels**
    c. Stage 1 (Hold)
        i. Reps: 1
        ii. Temp: 95°C
        iii. Time: 2:00
    d. Stage 2 (3-Step Amplification Stage)
        i. Highlight **step 3** and go to the lower left of the window and select **Insert Step** (ensure in the upper left of the window the drop-down menu for **Insert Step** has **After** selected).
        ii. Highlight **step 2** and set as follows:
            1. Temp: 92°C
            2. Time: 0:05
        iii. Highlight **step 3** and set as follows:
            1. Temp: 57°C
            2. Time: 0:05
            3. Go to the left of the screen and select **Remove Plate Read** button
        iv. Highlight **step 4** and set as follows:
            1. Temp: 68°C
            2. Time: 0:25
        v. Select **step 5**, the **GOTO step**, and leave the **GOTO step 2** but change the times to repeat to **14**
    e. Stage 3 (3-Step Amplification Stage)
        i. With step 5 highlighted select **Insert Step** button, on the lower left of the window, for a total of 3 times (until step 8 is reached)
        ii. Highlight **step 8** and select **Insert GOTO** button on the lower left of the window
        iii. Highlight **step 6** and set as follows:

1. Temp: 92°C
2. Time: 0:05

iv. Highlight **step 7** and set as follows:
1. Temp: 57°C
2. Time: 0:05

v. Highlight **step 8** and set as follows:
1. Temp: 68°C
2. Time: 0:25

vi. Select **step 9**, the **GOTO step**, and change to **GOTO step 6** and times to repeat to **34**

vii. Select step 8 and in the left of the window select **Add Plate Read to Step** button

f. Save the new cycling conditions as protocol for future use
i. At the upper left of the screen select the **Save** button
ii. Save in the **ExpressLoad** folder
iii. **Name** the file 'Quidel Molecular C. difficile'
iv. **Save as type** 'Protocol File (*.prcl)'
v. Select **Save**
vi. Click **Ok** in the protocol editor window

6. Define the plate setup
a. In the **Run Setup** window select the **Plate** tab
b. Under **Express Load** in the drop-down menu select **Quick Plate 96 wells All Channels.pltd**
c. Select the **Edit Selected** button to customize the plate setup
d. In the upper toolbar select **Settings.** The default settings need to be set.
i. **Plate Size** select **96 Wells**
ii. **Plate Type** select **BR Clear**
iii. **Number Convention** select **Scientific Notation**
iv. **Units** select **Copy Number**
e. Leave the **Scan Mode** set to **All Channels** at the top of the window
f. Select the **Select Fluorophores** button on the upper right of the Plate Editor window
i. De-select all default fluorophores
ii. Select **VIC** and **Cy5** and click Ok
g. In the **Plate Editor** window highlight the whole plate and click the check box in front of both **VIC** and **Cy5**
h. Select the **Experiment Settings** button in order to define the Targets
i. In the lower left of the **Experiment Settings** window in the **New** box type in **C. difficile** and select **Add**
ii. Repeat this for the **PRC**
iii. Select **Ok**
i. In the **Plate Editor** window next to **VIC** in the drop-down menu under **Target Name** select **C. difficile** and for Cy5 select **PRC**
j. Save the new plate setup for future use
i. At the upper left of the screen select the **Save** button
ii. Save in the **ExpressLoad** folder
iii. **Name** the file 'Quidel Molecular C. difficile plate'
iv. **Save as type** 'Plate File (*.pltd)'
v. Select **Save**
vi. Click **Ok** in the **Plate Editor** window
k. Exit the software

QUIDEL Molecular Direct

C. difficile

Supplemental Instructions: Analyzing a CFX96 Touch C. difficile Assay Run

Purpose: The following supplemental instructions will aid in analyzing a Quidel Molecular Direct C. difficile assay run on the CFX96 Touch from Bio-Rad. 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 Bio-Rad to ensure software compatibility.

Analysis Instructions:

1. Open the run file that needs to be analyzed
2. In the upper left select the **Quantification Tab**
3. On the Amplification curve check the box in front of **Log Scale**
4. Select **Settings** in the toolbar in the upper left of the screen
    a. For the **Cq Determination Mode** select **Single Threshold**
    b. Under the **Baseline Setting** choose **Baseline Subtracted Curve Fit**
    c. For **Analysis Mode** select **Target**
    d. Under **Cycles to Analyze** choose 1-35 and then click **Ok**
    e. The baseline cycles and the threshold for each target need to be set
        i. Ensure that only the ***C. difficile* box** is checked in the amplification plot
        ii. Go up to **Settings** in the toolbar and select **Baseline Threshold**
            1. At the top of the box select **Auto Calculated** for the **Baseline Cycles**
            2. For the **Single Threshold** at the bottom of the box select **User Defined**
                a. Set this to **34**
                b. Select Ok
        iii. **Uncheck** the ***C. difficile* box** and **check** the **PRC box** in the amplification plot
        iv. Go up to **Settings** in the toolbar and select **Baseline Threshold**
            1. At the top of the box select **Auto Calculated** for the **Baseline Cycles**
            2. For the **Single Threshold** at the bottom of the box select **User Defined**
                a. Set this to **50**
                b. Select Ok
5. Exit the software

Interpretation of the Quidel Molecular Direct C. difficile Assay Results on the CFX96 Touch			
Assay Result	**Detector: C. *difficile***	**Detector: Process Control**	**Interpretation of Results**
Negative	5.0 > Cq > 35.0	5.0 ≤ Cq≤ 35.0	No C. *difficile* DNA detected
C. *difficile* Positive	5.0 ≤ Cq ≤ 35.0	N/A	C. *difficile* DNA detected. Detection of PRC is not required when *C. difficile* is present.
Invalid	5.0 > Cq > 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.

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