Technical Bulletin

Technical Information for Quidel Molecular Direct C. difficile Assay

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Quidel Corporation has verified the performance of the Quidel Molecular Direct C. difficile Assay on Life Technology's 7500 Series of instruments (7500 Fast Dx, software version 1.4 and the 7500 Standard, software version 2.0). Internal studies performed by Quidel have demonstrated that by changing the threshold for the analytes the performance on these instruments is substantially equivalent to analytical and clinical performance data found in the package insert.

The cycling conditions for the 7500 Series of instruments are identical; however due to differences in the software versions there are additional programming procedures that should be followed for the 7500 Standard thermocycler. 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.

International Use Only

Direct C. difficile Assay

Supplemental Instructions: Creating an ABI 7500 Assay Protocol Template

Purpose: The following supplemental instructions will aid in programming an assay template for the 7500 from Life Technologies 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 Life Tech to ensure software compatibility.

Programming Instructions:

1. Launch the ABI 7500 software package.
2. Select the **Advanced Setup** button to open Setup and Experiment Properties. Follow each step to initiate the Quidel Molecular C. difficile protocol.
    a. Experiment Name: Enter Experiment Name as Quidel Molecular C. difficile. Leave the Barcode, User Name, and Comments fields blank
    b. Define Experiment Setup: Select 7500 (96 Wells), Quantitation- Standard Curve, TaqMan® Reagents, and Standard (~2 hours to complete a run)
3. In the upper left menu select **Plate Setup**
    a. Define Targets: New detectors for *C. difficile* and the process control (PRC) must be added.
        i. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
C. difficile	JOE	(none)	(Select)
PRC	Cy5	(none)	(Select)

        ii. Select **Add New Target** button for each target.
        iii. From each drop down menu select reporter, quencher, and color
        iv. Select a unique color to represent each detector
    b. Assign Targets and Samples: Under this tab in the bottom left corner, select **none** as the Passive Reference

4. Select **Run Method** from the upper left menu
    a. Set the **Reaction Volume** per Well to 20 µL under the **Graphical** or **Tabular View** and do not select expert mode
    b. Define the Thermocycler Protocol: Under the **Graphical** or **Tabular View** the default profile should be 2 holding stages and a 2-step cycling protocol. Each stage will have 3 user-editable text boxes. The first box value represents the Ramp Rate (%) for that stage, the second box value represents the temperature (°C) and the third box value represents the time (minutes:seconds).

        i. Make the following changes to the default Thermocycler protocol:
            1. Stage 1 **Holding Stage**
                a. Ramp Rate: 100%

b. Temp: 92
c. Time: 2:00

2. Highlight the second **Holding Stage** and select the **Delete Selected** button. Highlight Step 2 of the **Cycling Stage** and select **Add Step** button. In the drop down menu select **Before**.
3. First **3-Step Cycling Stage**
    a. Number of cycles: 15
    b. Do not check Enable Auto Delta
    c. Step 1
        i. Ramp Rate: 100%
        ii. Temp: 92
        iii. Time: 0:05
    d. Step 2
        i. Ramp Rate: 100%
        ii. Temp: 57
        iii. Time: 0:05
    e. Step 3
        i. Ramp Rate: 100%
        ii. Temp: 68
        iii. Time: 0:25
        iv. Turn data collection "Off" by selecting the **Data Selection** button just below the step time.
4. Highlight the **Cycling Stage** and select the **Add Stage** button. In the drop down menu select **Cycling**. Highlight Step 2 of the **Cycling Stage** and select **Add Step** button. In the drop down menu select **Before**.
5. Second 3-Step **Cycling Stage**
    a. Number of cycles: 35
    b. Do not check Enable Auto Delta
    c. Step 1
        i. Ramp Rate: 100%
        ii. Temp: 92
        iii. Time: 0:05
    d. Step 2
        i. Ramp Rate: 100%
        ii. Temp: 57
        iii. Time: 0:05
    e. Step 3
        i. Ramp Rate: 100%
        ii. Temp: 68
        iii. Time: 0:30
        iv. Ensure the data collection has been turned "On' for this step (default setting)
6. If a wrong stage is added the stage can be removed by pressing the **Undo "Add Stage"** button immediately after adding the stage or highlight the stage between the vertical lines and select the **Delete Selected** button

5. Set threshold for each analyte
    a. Set the **Analysis** tab in the upper left menu
    b. Select the **Amplification Plot** tab
    c. Select **Analysis Settings** button in the top right corner
    d. Highlight *C. difficile* and de-select the **Use Default Settings** box. De-select **Automatic Threshold** and change threshold to 40,000. Leave **Automatic Baseline** selected.
    e. Highlight PRC and de-select the **Use Default Settings** box. De-select **Automatic Threshold** and change threshold to 30,000. Leave **Automatic Baseline** selected.
    f. At the bottom of the box select **Apply Analysis Settings** button

Target	Threshold	Baseline Start	Baseline End
C. difficile	40,000	Auto	Auto
PRC	30,000	Auto	Auto

    g. Save the new protocol as a template for future use.
        i. At the top of the screen select the drop down menu next to **Save**
        ii. Choose **Save as Template**
        iii. Save in an appropriate folder
        iv. **File name:** 'Quidel Molecular C. difficile'
        v. **Save as type:** Experiment Document Template files (*.edt)'
    h. Exit the software.

6. Interpretation of results

Interpretation of the Quidel Molecular C. difficile Assay Results on the ABI 7500 Thermocycler			
Assay Result	**Detector: *C. difficile***	**Detector: Process Control**	**Interpretation of Results**
Negative	Ct < 5.0 or Ct > 35.0	5.0 ≤ Ct ≤ 35.0	No *C. difficile* DNA detected
C. difficile Positive	5.0 ≤ Ct ≤ 35.0	NA*	*C. difficile* DNA detected
Invalid	Undetermined, Ct < 5.0 or Ct > 35.0	Undetermined, Ct < 5.0 or Ct > 35.0	No *C. difficile* 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 Ct value is required for the Process Control to make a positive call.

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