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Molecular

Technical Bulletin

Technical Information for Quidel Molecular Influenza A+B Assay on 7500 Series

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Quidel Corporation has verified the performance of the Quidel Molecular Influenza A+B assay on Life Technologies' 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 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.

Internal studies demonstrate that the performance on the 7500 Standard is substantially equivalent to data found in the package insert when the Ct values used on the 7500 Fast Dx are applied. A new interpretation table for the results has been added on page 4.

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.

QUIDEL Molecular

Influenza A+B 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 Standard from Life Technologies 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 assay specifically. Its suitability for other assays is unknown. Check with Life Technologies 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 Influenza A+B protocol.
    a. Experiment Name: Enter Experiment Name as Quidel Molecular Influenza A+B. 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 Influenza A, Influenza B, and the process control (PRC) must be added.
        i. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
Influenza A	FAM	(none)	(Select)
Influenza B	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**
    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 (^{o}C) and the third box value represents the time (minutes:seconds).

        i. Make the following changes to the default Thermocycler protocol:
            1. Stage 1 First **Holding Stage**
                a. Ramp Rate: 100%
                b. Temp: 55
                c. Time: 5:00
            2. Step 1 Second **Holding Stage**.

a. Ramp Rate: 100%
b. Temp: 60
c. Time: 5:00

3. Highlight the second **Holding Stage** and select the **Add Stage** button. In the drop down menu select **Holding**
4. Step 1 **Third Holding Stage**
    a. Ramp Rate: 100%
    b. Temp: 65
    c. Time: 5:00
5. First **2-Step Cycling Stage**
    a. Number of cycles: 10
    b. Do <u>not</u> 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: 0:40
        iv. Turn data collection "Off" by selecting the **Data Selection** button at the bottom of the step.
6. Highlight step 2 and select the **Add Stage** button. In the drop down menu select **Cycling**
7. Second 2-Step **Cycling Stage**
    a. Number of cycles: 35
    b. Do <u>not</u> 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: 0:40
        iv. Ensure the data collection has been turned "On" for this step (default setting)
8. 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 Influenza A and deselect the **Use Default Settings** box. De-select **Automatic Threshold** and change threshold to 150,000. Leave **Automatic Baseline** selected.
    e. Highlight Influenza B and de-select the **Use Default Settings** box. De-select **Automatic Threshold** and change threshold to 120,000. Leave **Automatic Baseline** selected.

f. Highlight PRC and de-select the **Use Default Settings** box. De-select **Automatic Threshold** and change threshold to 27,000. Leave **Automatic Baseline** selected.

g. At the bottom of the box select **Apply Analysis Settings** button

Target	Threshold	Baseline Start	Baseline End
Influenza A	150,000	Auto	Auto
Influenza B	120,000	Auto	Auto
PRC	27,000	Auto	Auto

h. 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 Influenza A+B'
   v. **Save as type: '**Experiment Document Template files (*.edt)'

i. Exit the software.

Interpretation of the Quidel Molecular Influenza A+B Assay Results on the 7500 Thermocycler				
Assay Result	**Detector: Influenza A**	**Detector: Influenza B**	**Detector: Process Control**	**Interpretation of Results**
Negative	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	5.0 ≤ Ct ≤ 35.0	No Influenza A or Influenza B viral RNA detected; PRC Detected
Influenza A Positive	5.0≤ Ct ≤35.0	Ct<5.0 or Ct>35.0	NA*	Influenza A viral RNA detected
Influenza B Positive	Ct < 5.0 or Ct > 35.0	5.0 ≤ Ct ≤ 35.0	NA*	Influenza B viral RNA detected
Influenza A and B Positive	5.0≤ Ct ≤35.0	5.0≤ Ct ≤35.0	NA*	Influenza A and Influenza B viral RNA detected
Invalid	Undetermined, Ct < 5.0 or Ct > 35.0	Undetermined, Ct < 5.0 or Ct > 35.0	Undetermined, Ct < 5.0 or Ct > 35.0	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 Ct value is required for the Process Control to make a positive call.

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