



Technical Bulletin

Technical Information for Lyra Respiratory Panel Assay

Quidel Corporation has verified the performance of the Lyra Influenza A+B and the RSV + hMPV Assays individually on Life Technology's 7500 Fast Dx (software version 1.4). Internal studies performed by Quidel have demonstrated that when these two assays are tested on the same plate the performance is substantially equivalent to analytical and clinical performance data found in their respective Package Inserts.

For ease of use Quidel has combined these two protocols into one Respiratory Panel Assay. The cycling conditions for Influenza A+B and RSV + hMPV assays are identical; therefore these two assays can be performed at the same time to detect Influenza A, B, RSV, and hMPV. These procedures are detailed on pages 2-6. In order to detect all four analytes it is necessary to test each sample with both Lyra kits.

Please contact Quidel Technical Support at 800.874.1517 (in the U.S.), 858.552.1100 (outside the U.S.) or technicalsupport@quidel.com if you have any questions regarding the Lyra Influenza A+B Assay, or any Quidel product. Our hours of operation are Monday through Friday, 8:00 a.m. to 5:00 p.m. Eastern Time.

You may also visit our website at quidel.com for information on Quidel's line of Molecular Diagnostics, Rapid Diagnostics, Cell Culture and Specialty Diagnostics (Bone Health and Autoimmune & Complement). Other product information available on our website includes: CPT codes, CLSI procedure guides, MSDS, and Package Inserts.



Respiratory Panel Assay

Supplemental Instructions: Creating an ABI 7500 Fast Dx Assay Protocol Template

Purpose: The following supplemental instructions will aid in programming an assay template for the 7500 Fast Dx from Life Technologies to run the Lyra Influenza A+B and RSV+hMPV Assay kits. 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 the Lyra Influenza A + B and RSV + hMPV RT-PCR kits specifically. Its suitability for other assays is unknown. Check with Life Technologies to ensure software compatibility.

Programming Instructions:

1. Launch the ABI 7500 Fast Dx software package.
2. The **Quick Startup document** dialog window will open. Select the **Create New Document** button to start the **New Document Wizard**. Follow each step to initiate the Respiratory panel protocol.
 - a. Define Document: Most of the following should be the default setting. If not, change accordingly.
 - i. Confirm or enter the following information.

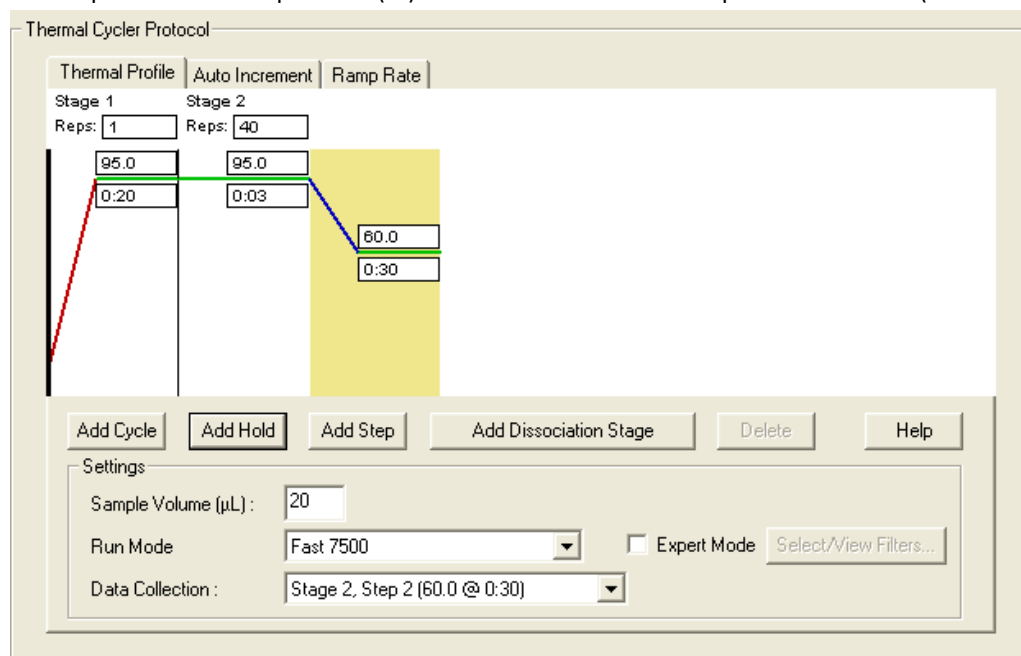
Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Blank Document
Run Mode:	Fast 7500
Operator:	<i>your operator name</i>
Comments:	SDS v1.4 (<i>add more if needed</i>)
Plate Name:	'Lyra Respiratory Panel'

- ii. Select the **Next** button.
- b. Select Detectors: New detectors for Influenza A, Influenza B, RSV, hMPV and the process control (PRC) must be added. For each target, select the **New Detector** button to open the **New Detector** pop-up window. Alternatively, use the **Create Another** button from within the **New Detector** pop-up window for the last four detectors.
 - i. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
Influenza A	FAM	(none)	(Select)
Influenza B	JOE	(none)	(Select)
RSV	FAM	(none)	(Select)
hMPV	Texas Red	(none)	(Select)
PRC	Cy5	(none)	(Select)

- ii. Select a unique color to represent each detector.
 - iii. Highlight the new detectors and add to the **Detectors in Document** column using the **Add** button.
 - iv. Select **(none)** from the **Passive Reference** drop-down menu.

- v. Select the **Next** button.
- vi. Select the **Finish** button without setting any wells.
- c. The wizard will close and the software will open, starting with the **Setup** tab. This will show the sample plate that was set up during the quick start. For the initial set up, nothing needs to be changed here.
- d. Defining the Thermocycler Protocol: Select the **Instrument** tab to set up the Respiratory Panel RT-PCR cycling times and temperatures. Under **Thermal Profile** there should be a default 2-stage protocol. Each stage will have 3 user-editable text boxes. The top box value represents the number of reps or cycles for that stage. The middle box value represents the temperature (°C) and the lowest box value represents the time (minutes: seconds).



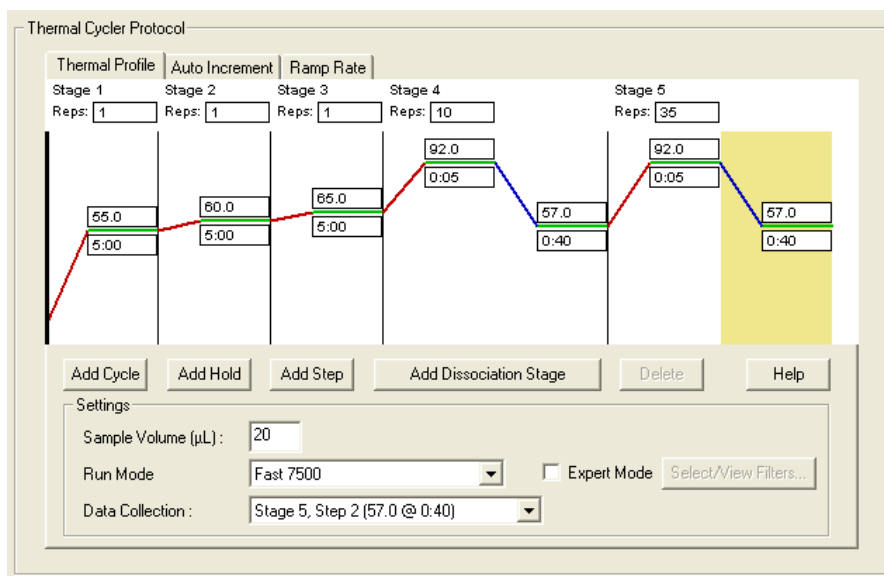
- i. Make the following changes to the default **Thermal Cycler Protocol**:
 1. Stage 1
 - a. Reps: 1
 - b. Temp: 55
 - c. Time: 5:00
 2. Select the bar between Stage 1 and Stage 2. Select the **Add Hold** button to add another stage.
 3. Stage 2
 - a. Reps: 1
 - b. Temp: 60
 - c. Time: 5:00
 4. Select the bar between Stage 2 and Stage 3. Select the **Add Hold** button to add another stage.
 5. Stage 3
 - a. Reps: 1
 - b. Temp: 65
 - c. Time: 5:00
 6. Stage 4 (2-Step Dissociation Stage)
 - a. Reps: 10
 - b. Step 1
 - i. Temp: 92
 - ii. Time: 0:05

- c. Step 2
 - i. Temp: 57
 - ii. Time: 0:40
7. Select the bar to the right of Stage 4. Select the **Add Cycle** button to add another stage.
8. Stage 5 (2-Step Dissociation Stage)
 - a. Reps: 35
 - b. Step 1
 - i. Temp: 92
 - ii. Time: 0:05
 - c. Step 2
 - i. Temp: 57
 - ii. Time: 0:40
9. If a wrong stage is added the stage can be removed by pressing the **Delete** button after highlighting the stage between the vertical lines

- ii. Under **Settings** enter the following:

Sample Volume (µL):	20 (default)
Run Mode:	7500 Fast (default)
Data Collection:	Stage 5, Step 2(57.0 @ 0:40)
NOTE: Do not check the check box next to 'Expert Mode'.	

- iii. Final protocol



- e. Set threshold for each analyte.
 - i. Select the **Results** tab.
 - ii. Select the **Amplification Plot** tab.
 - iii. Select Influenza A from the Detector tab in the top right corner.

- iv. In the **Analysis Settings** block, set the **Threshold** to **1.5e5**.
- v. Select the **Auto Baseline** radio button.
- vi. Repeat iii-v for Influenza B setting the **Threshold** to **1.2e5**.
- vii. Repeat iii-v for RSV setting the **Threshold** to **8.0e4**
- viii. Repeat iii-v for hMPV setting the **Threshold** to **5.4e4**
- ix. Repeat iii-v for PRC setting the **Threshold** to **2.7e4**.

- f. Save the new protocol as a template for future use.
 - i. At the top of the screen select **File** and then **Save As**.
 - ii. **Save In:** D:\Applied Biosystems\7500 Fast System\Templates\
 - iii. **File name:** 'Lyra Respiratory Panel'
 - iv. **Save as type:** 'SDS Templates (*.sdt)'
- g. Exit the software.

3. Interpretation of results

Interpretation of the Lyra Influenza A+B Assay Results on the 7500 Thermocycler						
Assay Result	Detector: Influenza A	Detector: Influenza B	Detector: RSV	Detector: hMPV	Detector: Process Control	Interpretation of Results
Negative	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	5.0 ≤ Ct ≤ 35.0	No Influenza A, Influenza B, RSV, or hMPV viral RNA detected; PRC Detected
Influenza A Positive	5.0 ≤ Ct ≤ 35.0	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or 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	Ct < 5.0 or Ct > 35.0	Ct < 5.0 or Ct > 35.0	NA*	Influenza B viral RNA detected

Interpretation of the Lyra Influenza A+B Assay Results on the 7500 Thermocycler						
Assay Result	Detector: Influenza A	Detector: Influenza B	Detector: RSV	Detector: hMPV	Detector: Process Control	Interpretation of Results
Influenza A and B Positive	$5.0 \leq Ct \leq 35.0$	$5.0 \leq Ct \leq 35.0$	$Ct < 5.0$ or $Ct > 35.0$	$Ct < 5.0$ or $Ct > 35.0$	NA*	Influenza A and Influenza B viral RNA detected
RSV Positive	$Ct < 5.0$ or $Ct > 35.0$	$Ct < 5.0$ or $Ct > 35.0$	$5.0 \leq Ct \leq 35.0$	$Ct < 5.0$ or $Ct > 35.0$	NA*	RSV viral RNA detected
hMPV Positive	$Ct < 5.0$ or $Ct > 35.0$	$Ct < 5.0$ or $Ct > 35.0$	$Ct < 5.0$ or $Ct > 35.0$	$5.0 \leq Ct \leq 35.0$	NA*	hMPV viral RNA detected
RSV and hMPV	$Ct < 5.0$ or $Ct > 35.0$	$Ct < 5.0$ or $Ct > 35.0$	$5.0 \leq Ct \leq 35.0$	$5.0 \leq Ct \leq 35.0$	NA*	RSV and hMPV 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 $Ct > 35.0$	Undetermined, $Ct < 5.0$ or $Ct > 35.0$	Undetermined, $Ct < 5.0$ or $Ct > 35.0$	No Influenza A, Influenza B, RSV, or hMPV 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.