

Appendix 6: ABI 7500 Fast v1.4 Method (1 of 7)

(A) Define Protocol as a Template

Turn on the ABI 7500 FAST Real-Time PCR instrument and computer. Open 7500 Fast System Software v1.4. It shows “Quick start” →click “cancel”. From “File”→ New→ New Document Wizard (Define Document) → “Next” → “Select Detectors” → “Next” → “Set Sample Plate” → “Finish” → Click on the “Instrument” tab.

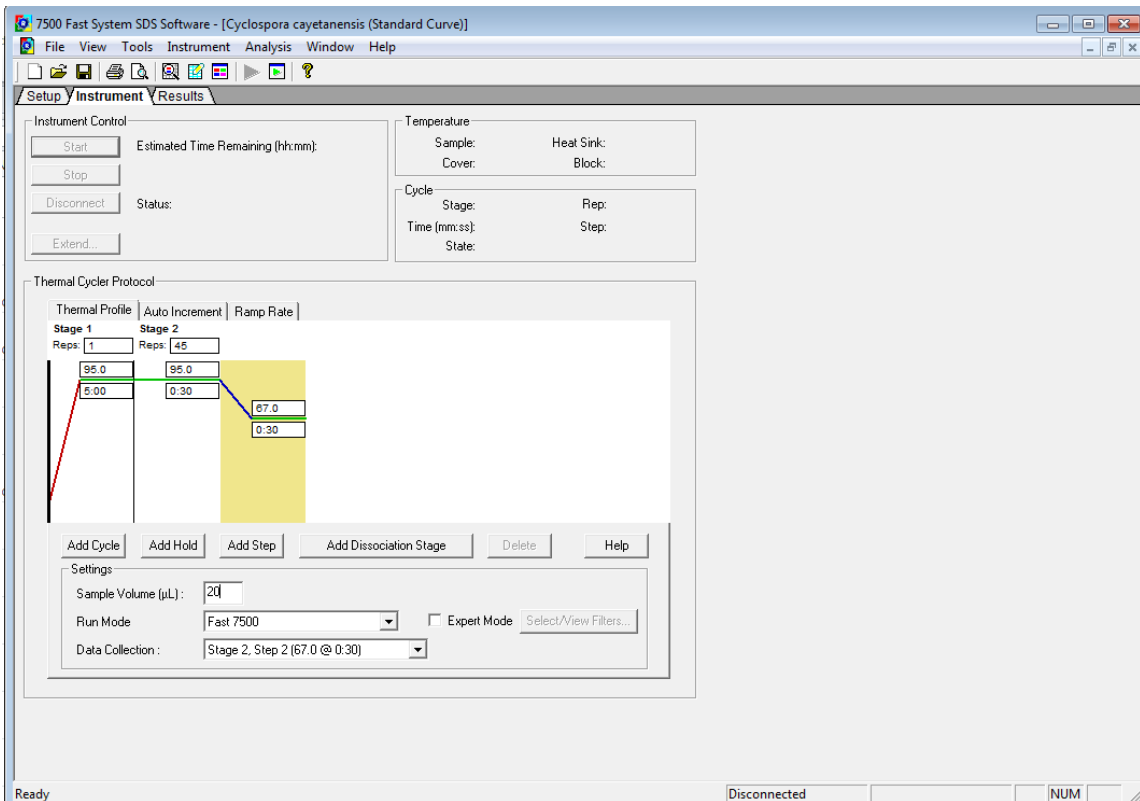
1. Key in the following parameters:

Stage 1: Reps:1
95 °C; 5:00
Stage 2: Reps: 45
95 °C; 0:30
67 °C; 0:30

2. Sample Volume (µL): **20**

Run Mode: **Fast 7500**

Data collection: **Stage 2, step 2 (67.0@ 0:30)**

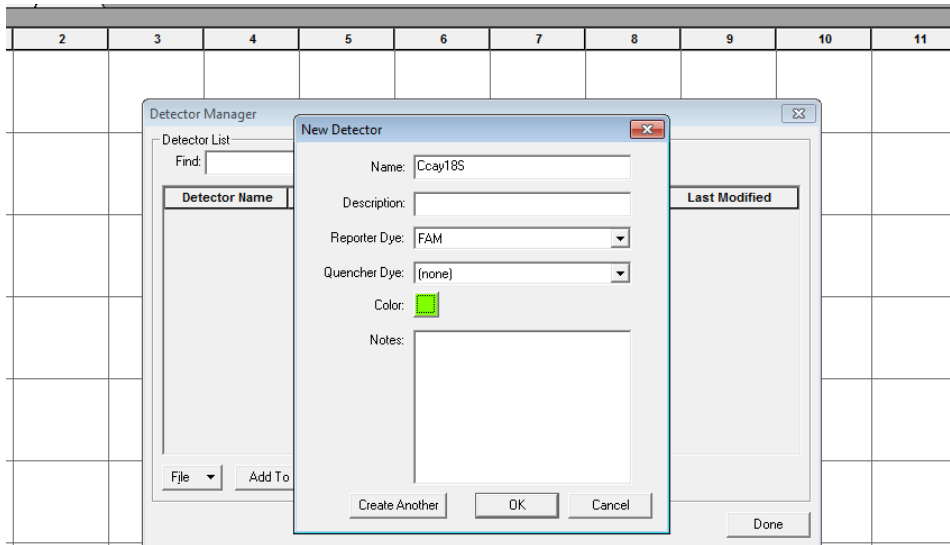


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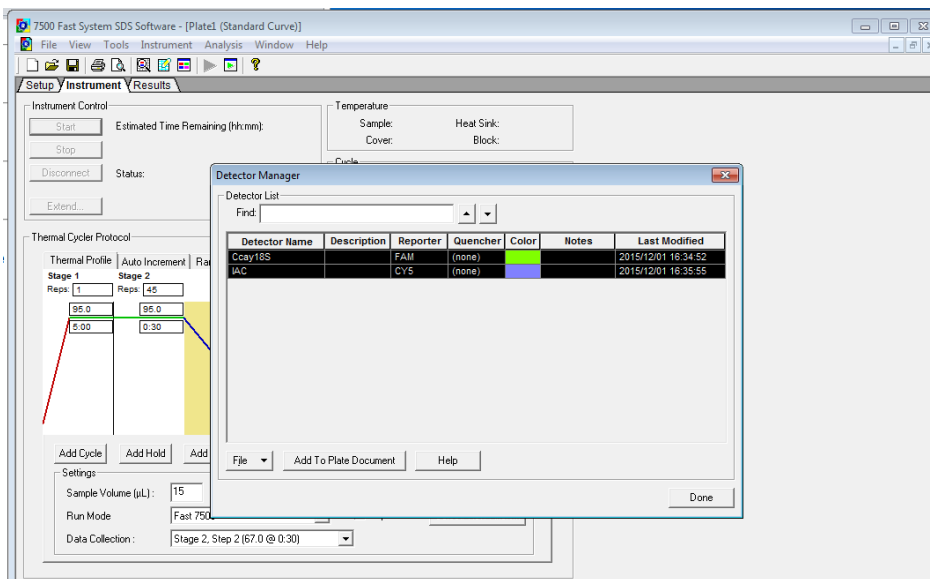
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(B) Create Detectors: *Ccay18S* & IAC (internal amplification control)

Select “Tools” → “Detector Manager” → File → “New” → “Name”: **Ccay18S**, “Reporter Dye: **FAM**, “Quencher Dye”: None, “Color”: Green →OK. Click “Create Another” →File → New→ “Name”: **IAC**, Reporter Dye: **Cy5**, “Quencher Dye”: None, “Color”: Purple → “OK”. Click “OK” on “New detector” window.



Highlight both the Ccay18S and IAC detectors. Click “Add to Plate document”. Click “OK” after each detector is added to plate. Click “Done” on “Detector Manager Window”.



Save Protocol as a SDS Template [*sdt] in Drive D: →Applied Biosystems →7500 system → templates→ File Name: “Cyclospora cayetanensis”.

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(C) Start a new Run

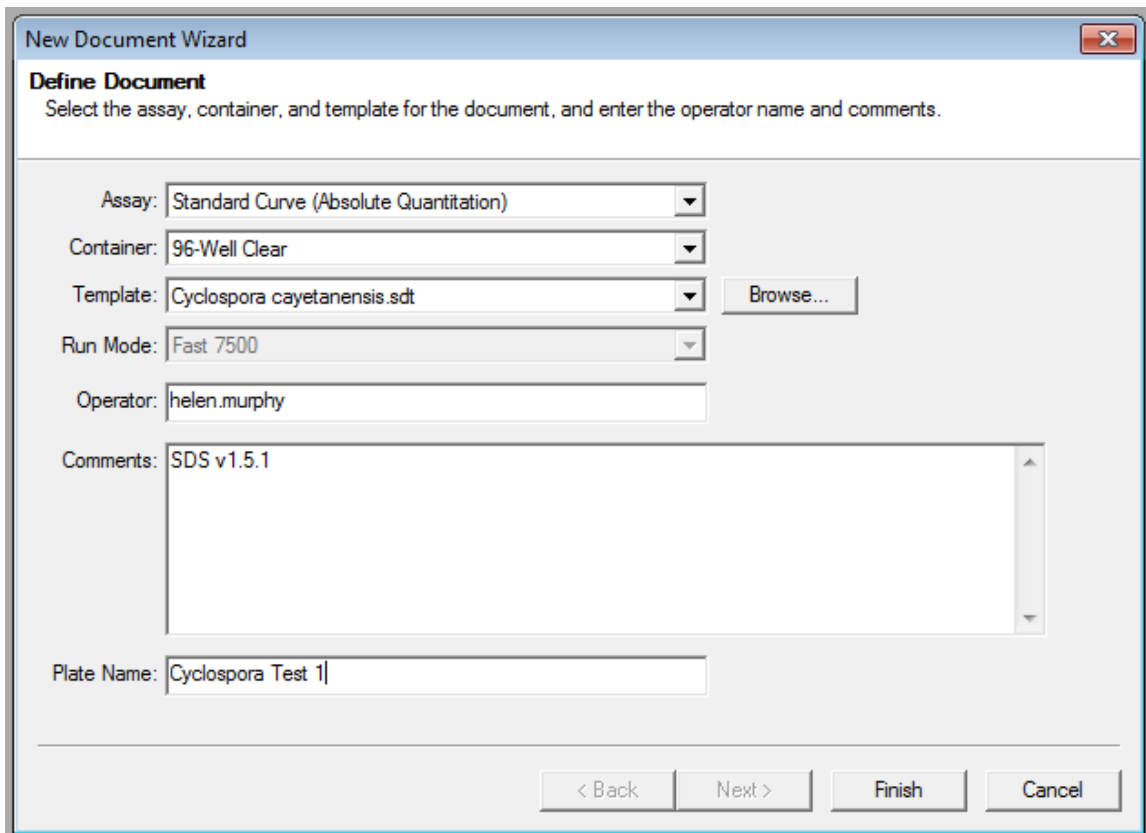
Turn on the ABI 7500 FAST Real-Time PCR instrument and computer. Open 7500 Fast System Software v1.4.

From “Quick Startup” → Click “Create New Document” to open “New Document Wizard” and “Define Document”.

Assay: Standard Curve (Absolute Quantitation)

Template: Click on “Browse” → navigate to “templates” folder → Select “Cyclospora cayetanensis.sdt”

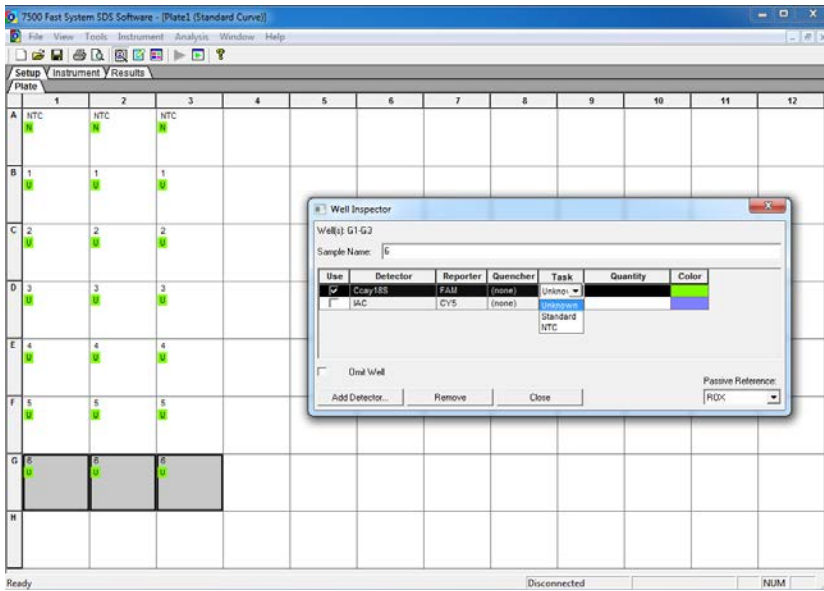
Plate Name: “Cyclospora Test 1” → “Finish”



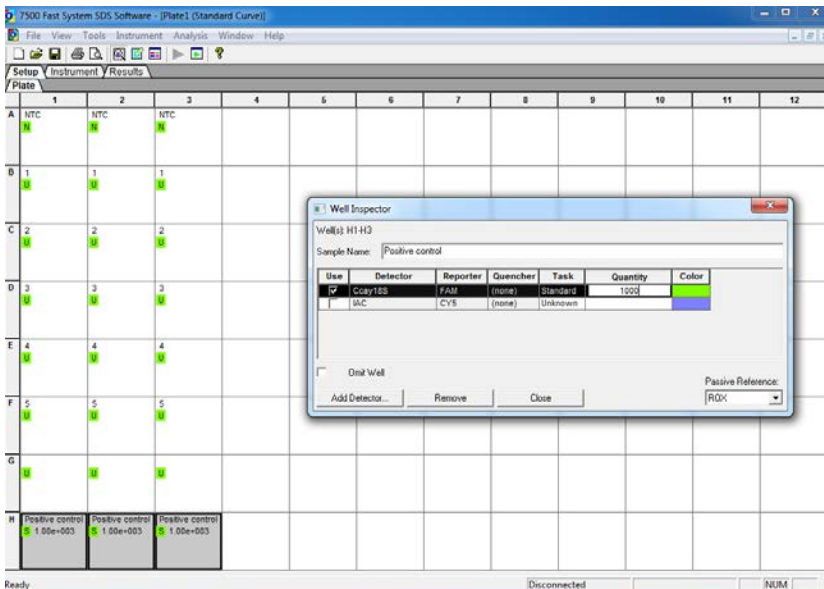
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From “View” → Select “Well Inspector” to define well assignments. Define the no template control wells by selecting three wells and type “NTC” in the “Sample Name” field on the “Well Inspector” window. Check the “Ccy18S” detector choosing “NTC” as “Task”. Define all unknown samples and DNA extraction control samples in the same manner but choose “Unknown” as “Task”.



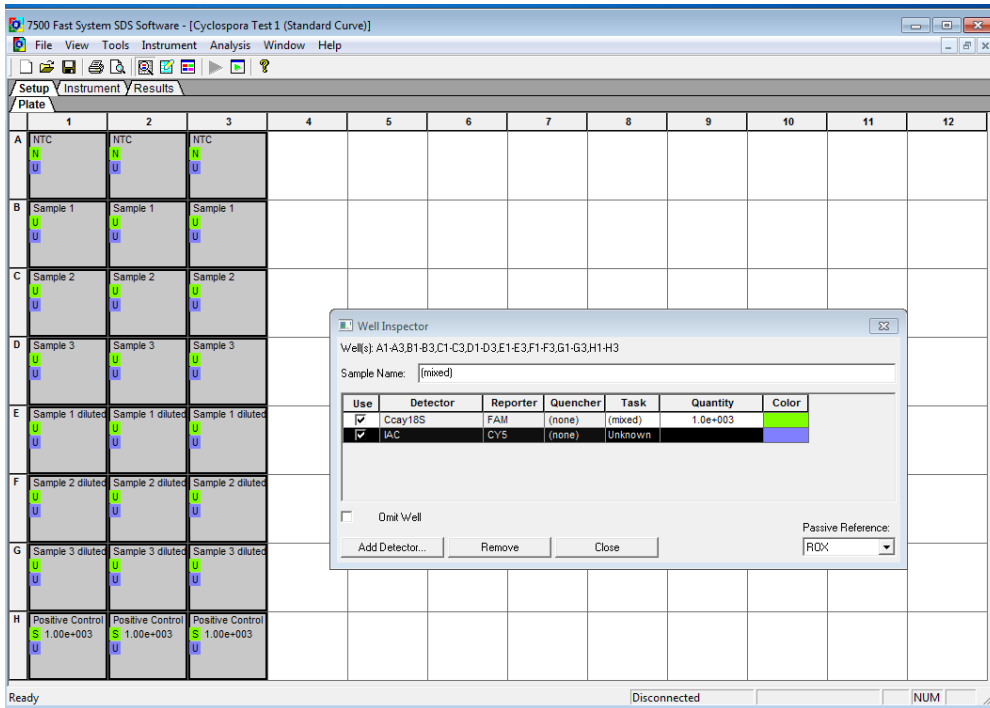
Define the positive control in the same manner but choose “Standard” as “Task” and enter “1000” as “Quantity”.



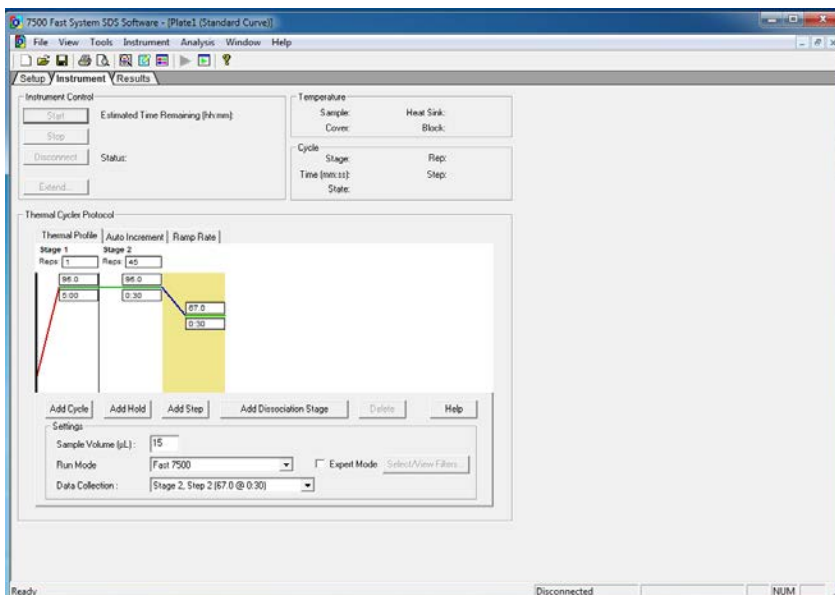
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Assign the IAC detector to all wells by highlighting all reaction wells: Check the “IAC” detector in the “Well Inspector” window and choose “Unknown” as “Task”. Close the “Well Inspector” window. From the “File” menu → “Save” to choose a location to save the run file.



Insert the plate to begin the run and click the “Instrument” tab → “Start”.



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(D) Results

Click on the “Results” tab → Select the “Amplification Plot” tab. Select all wells in the lower portion of the window. On the “Data” drop down menu on the right select choose “**Delta Rn vs. Cycle**”, and then define Analysis Settings for each target:

Select Detector “**Ccay18S**” and Line Color “**Detector Color**”. In the “Analysis Settings” box select:

“**Manual Ct**” Threshold = **0.02**

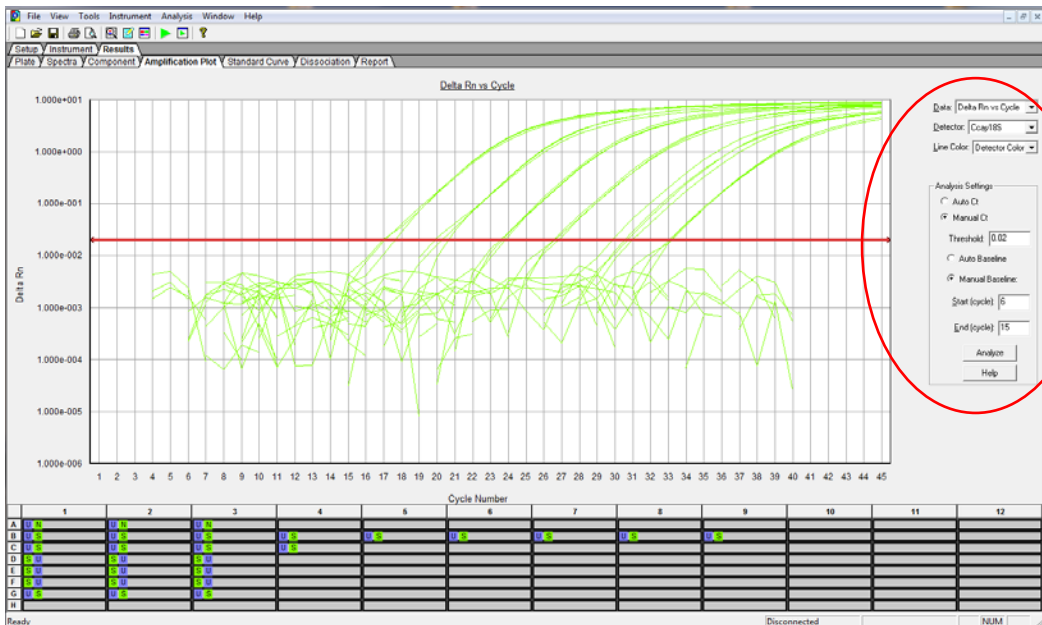
“**Manual Baseline**” Start = **6**, End = **15**

Select Detector “**IAC**” and Line Color “**Detector Color**”. In the “Analysis Settings” box select:

“**Manual Ct**” Threshold = **0.01**

“**Manual Baseline**” Start = **6**, End = **15**

Click “**Analyze**”. Save the run analysis results as an SDS Document (*.SDS).



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Click on the “Report” tab. Assure that all wells are still selected in the lower portion of the window.

Open a window to define the report settings by clicking the “Report Settings” icon.

Choose Data Columns:
Well
Sample Name
Detector
Task
Ct
StdDev Ct

Uncheck all data boxes on the right side of the window.

Click OK on the report settings window.

Review the Amplification Plots and Ct’s for each target. Verify that all criteria for a valid experimental run are met as defined in the “Interpretation of Results” section of the protocol.

Save an **Experimental Results Report** by clicking the “File” drop down menu → “Export” → “Results”. On the “Select Results Export File” window choose a location to save the results file.

File name: *use experiment run name.*

Save as type: *Results Export Files (*.cvs).*

Click “Save”. Check both options on the “Export Settings” window that appears → click OK.