

# Protocol Sheet

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## ABI 7500<sup>®</sup> & ABI 7500 FAST<sup>®</sup> (Software Version 2.0.4 instrument setup instructions for RT<sup>2</sup> Profiler PCR Arrays

### Important points before starting

- Please read the handbook supplied with the RT<sup>2</sup> Profiler PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

### Procedure

#### Creation of PCR protocol template

1. Open the ABI 7500 software (V. 2.0.4) on the desktop of the computer that is connected to the ABI 7500 (Standard or FAST) system.
2. Select New Experiment from top left of the screen.

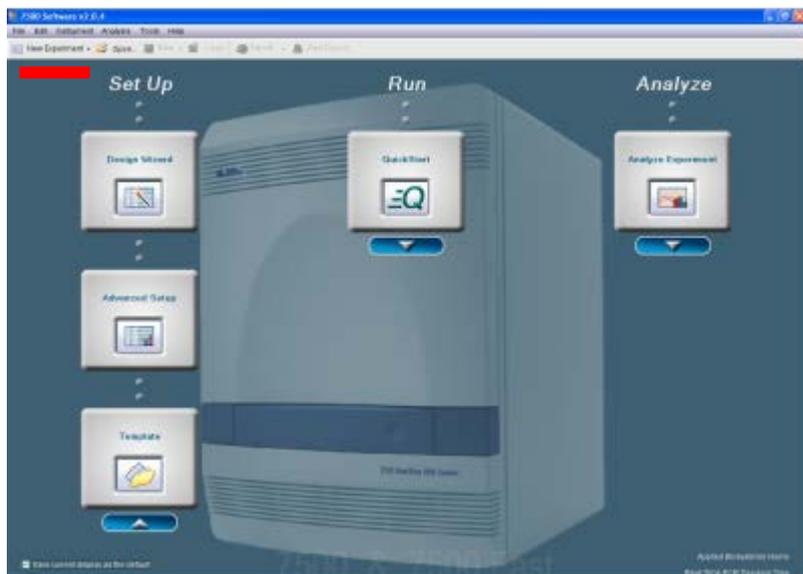


Figure 1. New experiment setup screen for ABI 7500 software version 2.0.4.

#### 3. Define Experiment Properties

- How do you want to identify this experiment?
  - Type in Experiment Name based on your experiment arrangement.
  - Type In Barcode, User Name, Comments (optional)



- Which instrument are you using to run the experiment?
  - Select 7500 (96 Wells) or 7500 FAST (96 Wells)
- Which type of experiment do you want to set up?
  - Select Quantification-Standard Curve
- Which reagents do you want to use to detect the target sequence?
  - Select SYBR® Green Reagents
- What ramp speed do you want to use in the instrument run?
  - Select Standard (about 2 hours to complete a run)

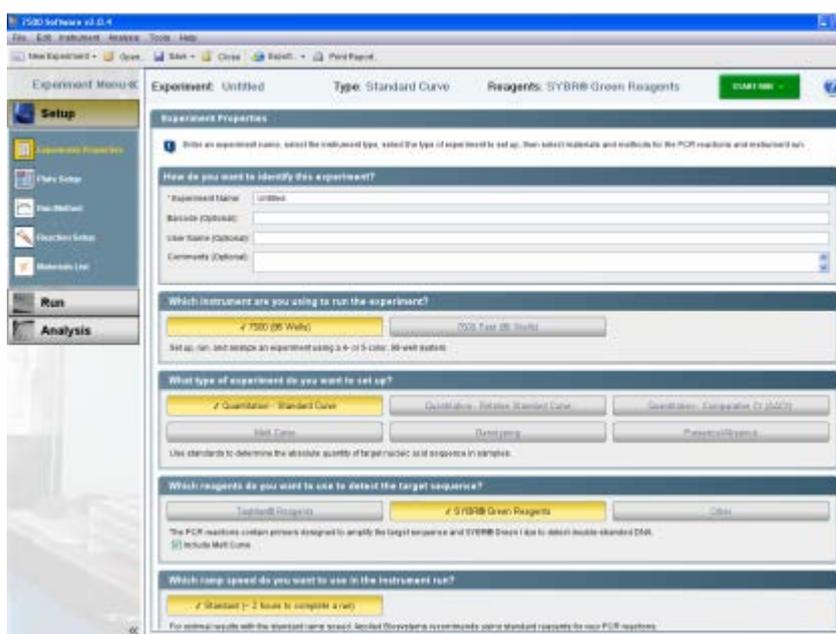


Figure 2. Experiment Properties screen.

#### 4. Click Plate Setup (from tab list on left)

- Click on Define Targets and Samples:
  - Target Name, use default Target 1; Reporter, select SYBR; Quencher, select None; Color, select desired color
  - Define Samples: select sample such as Sample 1 or your defined sample name.
  - Define Biological Replicate Group-None
  - Click Assign Targets and Samples

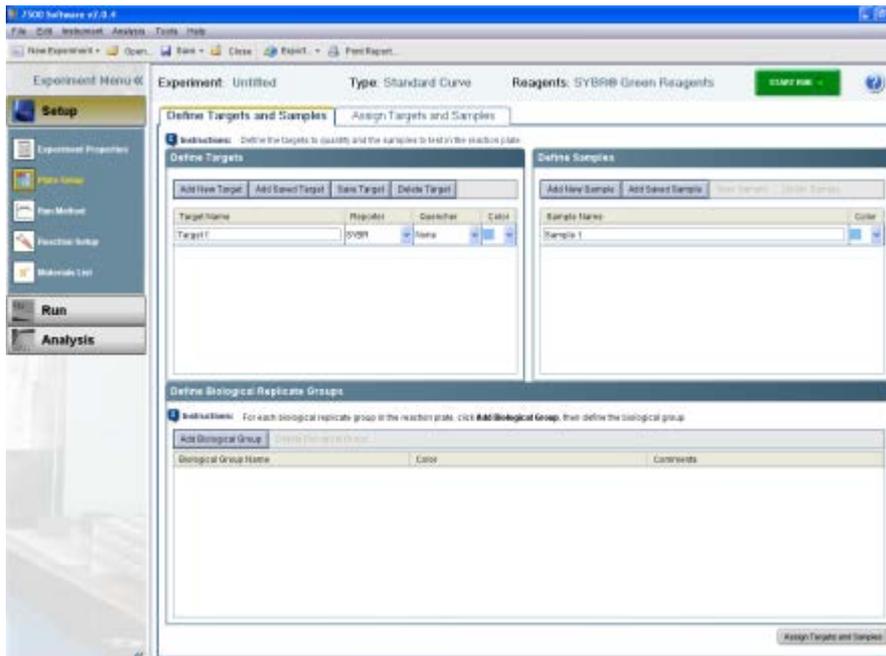


Figure 3. Assign Targets screen.

- Click Assign Targets and Samples tab
  - Highlight the entire plate by clicking the square between A and 1 in View Plate Layout.
  - Check the box next to Target 1 under Assign Targets to the Selected Wells (if you set different wells for different targets based on PCR Array format, you can check them individually)
  - Assign sample(s) to the selected wells. Select the Sample 1 or the name as defined before.
  - Verify all wells in Plate Layout view have the “U” symbol (“U” = unknown) and Sample 1.
  - Select the dye as the passive reference — select ROX.

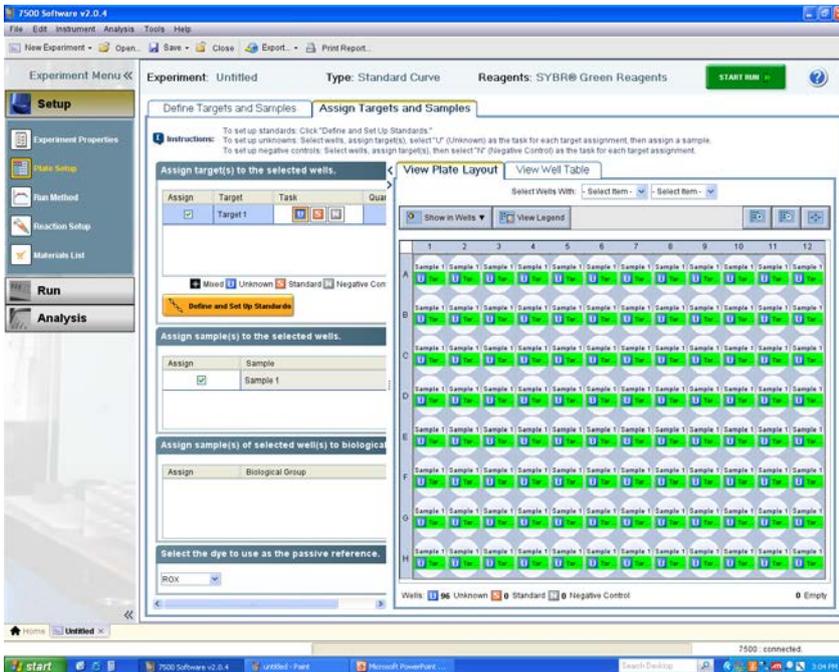


Figure 4. Assign Targets and Samples tab.

5. Click Run Method. This setting should default to run protocol with melting curve.
6. Change setting at Graphical view
  - Set Reaction Volume Per Well to 25  $\mu$ l
  - Holding Stage: 95.0°C 10:00 minutes
  - Cycling Stage:
    - Number of Cycles: 40
    - Temperature: 95.0°C 00:15; 60.0°C 01:00
  - Melting Curve Stage: Use default setting from 60°C to 95°C

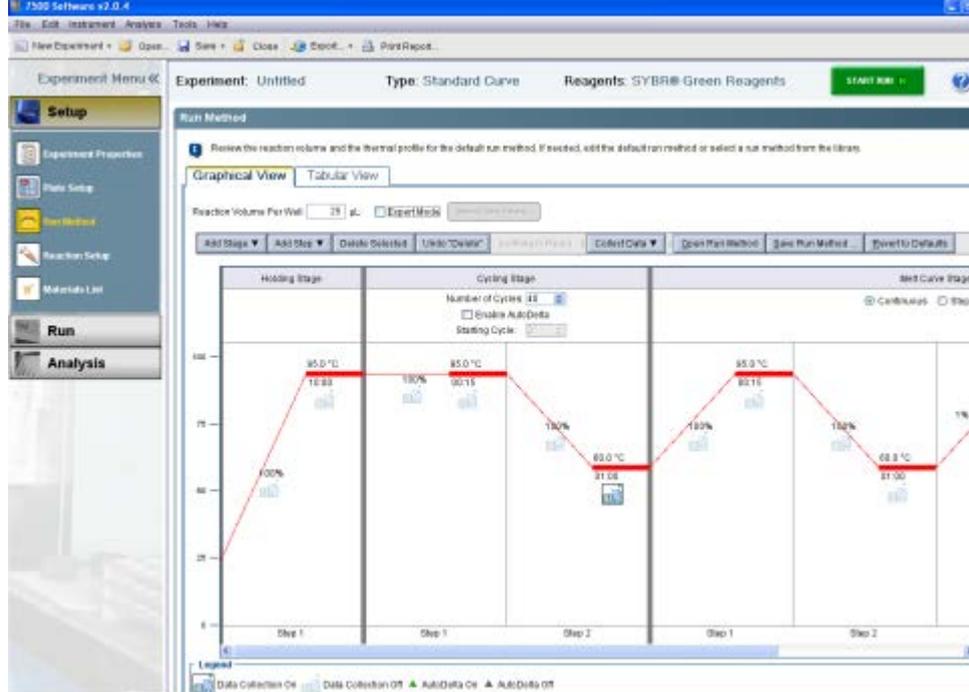


Figure 5. Graphical View screen.

## 7. Save template

- Click Save As Template under the File tab to save template file for further use. Alternatively, download a template from our website, <http://www.sabiosciences.com/pcrarrayprotocolfiles.php>

## Performing real-time PCR detection

- If the thermocycler is off, press the power button to switch on the instrument. Wait for the instrument to boot and display the Power status light. Switch on the computer connected to the thermocycler.
- Make sure that there are no bubbles in the wells, and that the reaction mix is positioned at the bottom of each well. If not, centrifuge the plate at ~1000 g for 60 seconds.
- Open the ABI 7500 Software v2.0.4 (or similar version)
- Select File>New Experiment>From Template. Select the right template for your experiment and click Open. This will load the previously saved plate setup to the new plate document.
- Add an Experiment Name in Setup>Experiment Properties.
- Save the new document under a new filename as Experiment Documents Single File (\*.sds).
- Load your plate in the plate tray with A1 in the top left corner.
- Select the Run tab on left panel. Click Start Run to begin the PCR run. Wait 30 seconds to 1 min for the initial priming, then the run should start. The estimated run time will then appear on the screen.

## After the PCR run

16. Select Analysis on the left panel. On the lower panel Options, select the radio button next to Auto Baseline and deselect the radio button next to Threshold: Auto. Add a desired threshold (most of time 0.05 is fine, please refer to step 20 for manual selection of the threshold). Click the Green Analyze button on the top right to do the data analysis.
17. Select the radio button on lower panel next to Threshold to show the threshold in figure. Do the same for Baseline information.
18. Under Amplification Plot/Plot Setting, default setting for Plot Type is  $\Delta Rn$  vs. Cycle. Other Plot Types may also be selected. For Graph Type, the default is Log format, but linear may also be selected.
19. For Exporting the results, Select File>Export. The Export Data window will appear. Under Export Properties:
  - Select data to export. Use the radio button to select the data you want to export, usually Results.
  - Select one file or separate file by selecting the radio button.
  - Enter export file properties for its name and location. Also select the File Type (choose \*.xls for Excel® file and \*.txt for regular text file).
  - Select the radio button beside Open file(s) when export is complete for view file immediately after exporting.

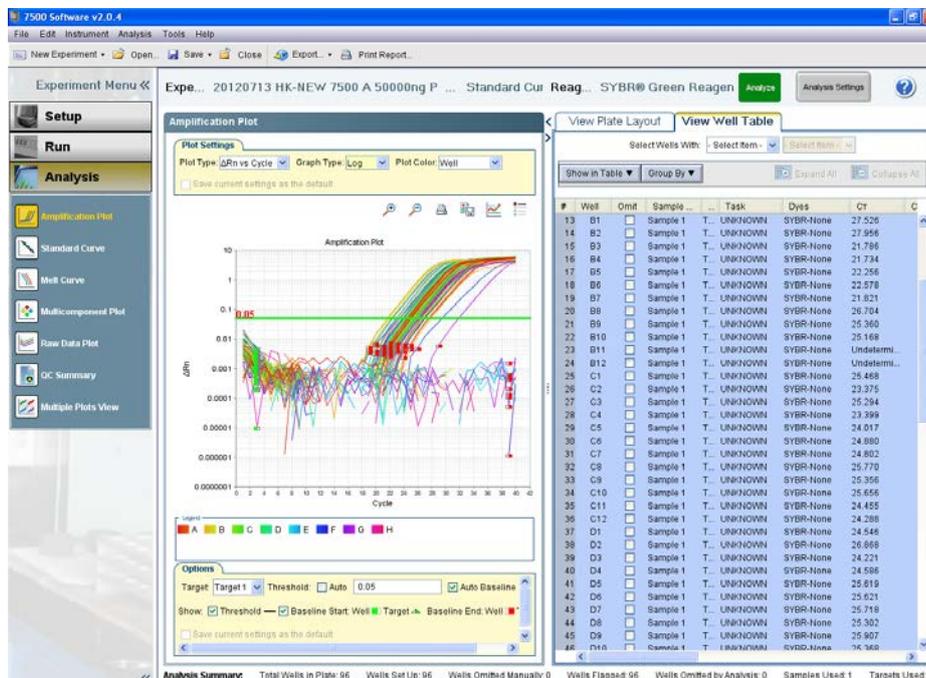


Figure 6. Data analysis screen.

**20. Follow the procedures below to select the baseline and threshold manually if needed:**

**Note: Please use the same threshold for all your plates in one study.**

- To define the Baseline, use the Linear View of the Amplification plot/Plot Settings. With the linear plots, determine the cycle number at which the earliest amplification can be seen. Using the red sliding bars on X-axis to set the Manual Baseline to start from cycle number 2 through two cycle values before the earliest visible amplification.
- To define the Threshold Value, use the Log View of the amplification plot/Plot Settings. With the log plots, place the threshold line above the background signal but within the lower third of the linear phase of the amplification plot (please remember the Threshold when you first time set it and use it for all your analysis in same study).

The RT<sup>2</sup> Profiler PCR Array is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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