# Protocol Sheet

## ABI 7900HT® (for SDS Software 2.1, 2.3 and 2.4) instrument setup instructions for RT² Profiler PCR Arrays

## Important points before starting

- Please read the handbook supplied with the RT² Profiler PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. The RT² Profiler PCR Array is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

- Please make sure the system has been properly calibrated for the region of interest (ROI), background, and pure dye. (Refer to the manufacturer's Installation and Maintenance manual for the calibration procedures if needed).

- Please make note of the installed SDS Software version on your instrument.

- Set up a PCR protocol template file on the ABI 7900HT Fast System SDS software as follows.

## Procedure

### Creation of PCR protocol template

1. **Open the ABI 7900 Fast System SDS Software on the desktop of the computer that is connected to the ABI 7900HT system.**

2. **Select File ➤ New, the New Document dialog box will appear.**

   - **Select Assay ➤**
     - **SDS 2.1: Absolute Quantification (Standard Curve)**
     - **SDS 2.3 : Standard Curve (AQ)**
     - **SDS 2.4 : Standard Curve (AQ)**
   - **Select Container ➤**
     - **SDS 2.1: 384-well Clear Plate**
     - **SDS 2.3: 96 or 384 wells Clear Plate**
     - **SDS 2.4: 96 or 384 wells Clear Plate**
   - **Select Template ➤**
     - **SDS 2.1: Blank Document**
     - **SDS 2.3: Blank Template**
     - **SDS 2.4: Blank Template**
   - **The field for Barcode is optional.**

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- ▪ **Click OK**
- ▪ **Under the Setup tab on the center-right, select Add Detector button from bottom.**
- ▪ **In the Detector Manager window,**
  - • **SDS2.1**
    - o **Select SYBR® for the reporter dye**
    - o **Click Copy To Plate Document button**
    - o **Click Done button (See Figure 1).**
    - o **Choose ROX for the Passive Reference box.**
  - • **SDS2.3**
    - o **Click "New"**
    - o **In the Add Detector Dialog Box,**
      - ▪ **Enter: SYBR in the "Name" Field**
      - ▪ **Select SYBR for the Reporter**
      - ▪ **Click OK**
    - o **Click once on the newly added Detector**
    - o **Click Copy To Plate Document button**
    - o **Click Done button**
    - o **Choose ROX for the Passive Reference box.**
  - • **SDS2.4**
    - o **Click "New"**
    - o **In the Add Detector Dialog Box,**
      - ▪ **Enter: SYBR in the "Name" Field**
      - ▪ **Keep "Group" field as Default**
      - ▪ **Keep "Description" and "AIF Assay ID" empty**
      - ▪ **Select SYBR for the Reporter**
      - ▪ **Select Non Fluorescent as Quencher**
      - ▪ **Click the square with color beside "Color", then select your preferred color in Select Detector Color/Swatches page (usually Green for SYBR). Click OK to close that page.**
      - ▪ **Click OK**
    - o **Click once on the newly added Detector**
    - o **Click Copy To Plate Document button**
    - o **Click Done button**
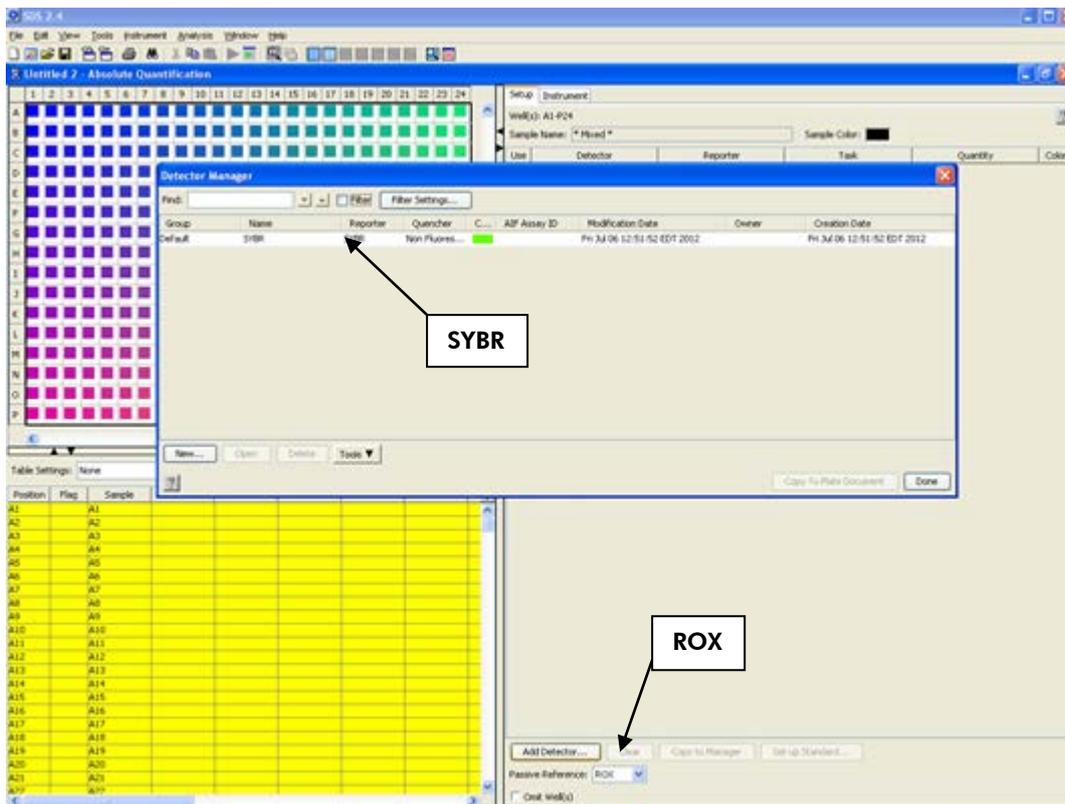    - o **Choose ROX for the Passive Reference box.**

**Figure 1. Plate setup — dye selection.**

- Select the Instrument tab, then the Thermal Profile tab. Highlight Stage 1 (50°C for 2:00 minutes) by right clicking the mouse and dragging it across Stage 1. Delete Stage 1 by clicking the Delete button. Then continue to edit the Thermal Profile as follows: Enter 95.0°C for 10:00 minutes for Stage 1; for Stage 2, enter 95.0°C for 0:15 (15 seconds) followed by 60.0°C for 1:00 minute with Repeats 40 (40 cycles). Click the Add Dissociation Stage button; the pre-set dissociation stage will be added as Stage 3.

  - For SDS Software 2.1: Uncheck the box next to "9600 Emulation".

  - For SDS Software 2.3/2.4: select "Standard" mode.

- Click Add Dissociation Stage for dissociation curve detection.

- Put 10 µl for Sample Volume.

- Then select File ➤Save As to save the template file. Save the file as SDS 7900HT Template Document (*.sdt) with the filename "RT2 *Profiler* PCR Array Protocol 7900HT Template" (click Save).

- Alternatively, download the PCR protocol template file (RT2*Profiler*™ PCR Array Protocol Template-ABI7900.sdt) from SABiosciences website (http://www.SABiosciences.com).
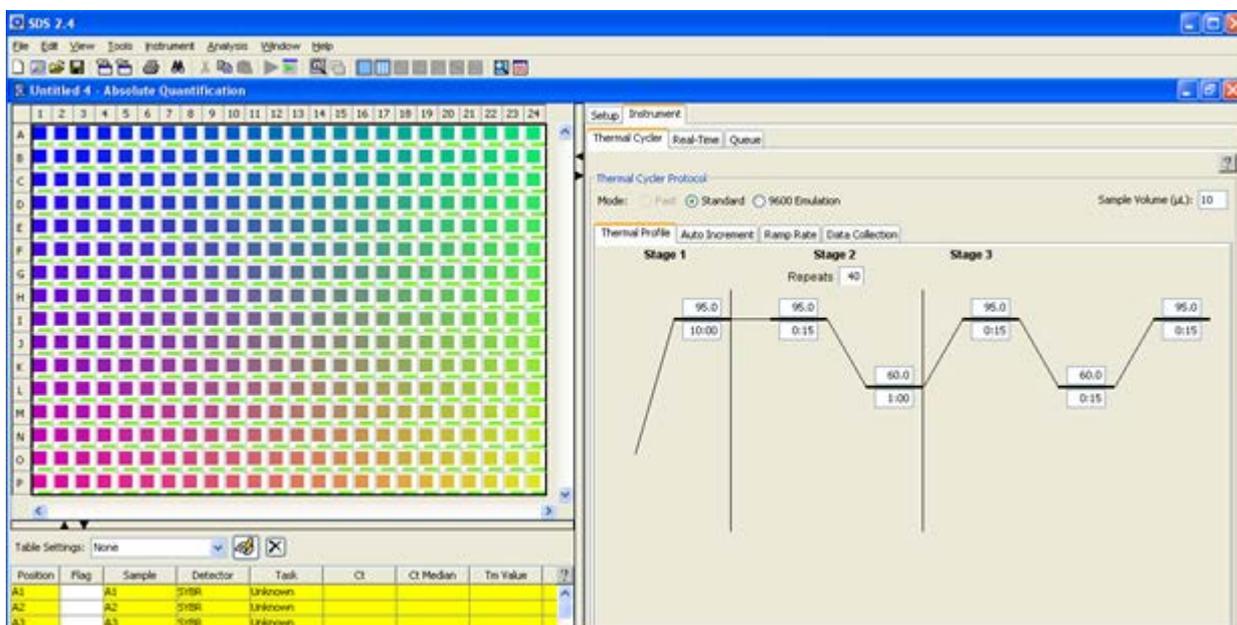
Figure 2. Plate setup — setting thermal profile.

## Performing real-time PCR detection

3.  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.

4.  Make sure that there are no bubbles in any well, and that the reaction mix is positioned at the bottom of each well. If not, centrifuge the plate at ~1000 g for 60 seconds.

5.  Open the ABI 7900HT Fast System SDS Software

6.  Select File ➤ New. In the New Document Wizard dialog box, select Browse to load the RT2 *Profiler* PCR Array Template file. Then click Finish. This will load the previously saved setup to the new plate document.

    - To set up a sample plate (See Figure 2), click the square button in the upper left corner of the full plate layout panel (between the letter "A" and "1") to select all wells. Once selected, the lower full plate table panel will be highlighted in yellow and the box next to "SYBR" on the left panel will be checked.

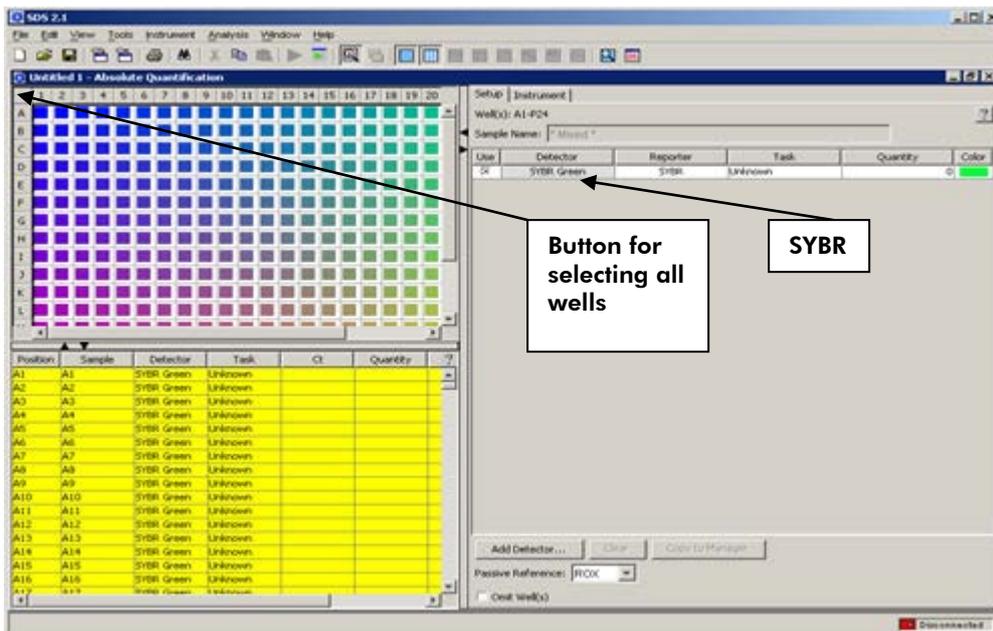7.  Save the new document under a new filename as SDS 7900HT Documents (*sds).

**Figure 3. Plate setup — selecting sample.**

8. Select the Instrument/Real-Time tab. Click Connect/Connect to Instrument to connect the computer to the thermal cycler. Click Open to open the plate tray and place your plate in the precision plate holder with A1 in the top left corner. Click Close to load the plate. Click Start/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

9. When the PCR run is complete, a small dialog box stating "The run completed successfully" will appear on the screen. Click OK, this will close the box.

10. Select Analysis ➤ Analysis Settings, the Analysis Settings dialog box will appear. Select the radio buttons next to Automatic Baseline and Manual $C_T$ (most of time 0.2 is fine, please refer 5) for manual selecting threshold), click OK.

11. Click the Result tab. Display Data as $\Delta$Rn vs. Cycle. If you have not selected all wells, click the square button in the upper left corner of the diagram of the plate (between the letter "A" and "1") to select all wells. The selected wells will be highlighted in yellow in the lower left panel.

12. Select Analysis ➤ Analyze. It will calculate the data and generate the $C_T$ (lower-left panel).

13. Follow the procedures below to select the baseline and threshold manually (see Figures 4 and 5):

   ▪ (Please use the same threshold for all your plates in one study).

- To define the Baseline, use the Linear View of the amplification plots. Double click on Y-axis. The window for Display Settings will appear. For Amplification Plot Properties, select Auto Scale for both the Y and X-Axes. Select Linear view for Y-Axis, then click OK. 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 plots. Double click on Y-axis. The window for Display Settings will appear. For Amplification Plot Properties, select Auto Scale for both the Y and X-Axes. Select Log view for Y-Axis and click OK. 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.

- The values for $C_T$ will be displayed in the lower left panel for each well. To export the result to an Excel® spreadsheet, select File ➤Export ➤Results Table and save the file as Tab-delimited Text file (*txt). This file format can be opened in the Microsoft Excel Program.
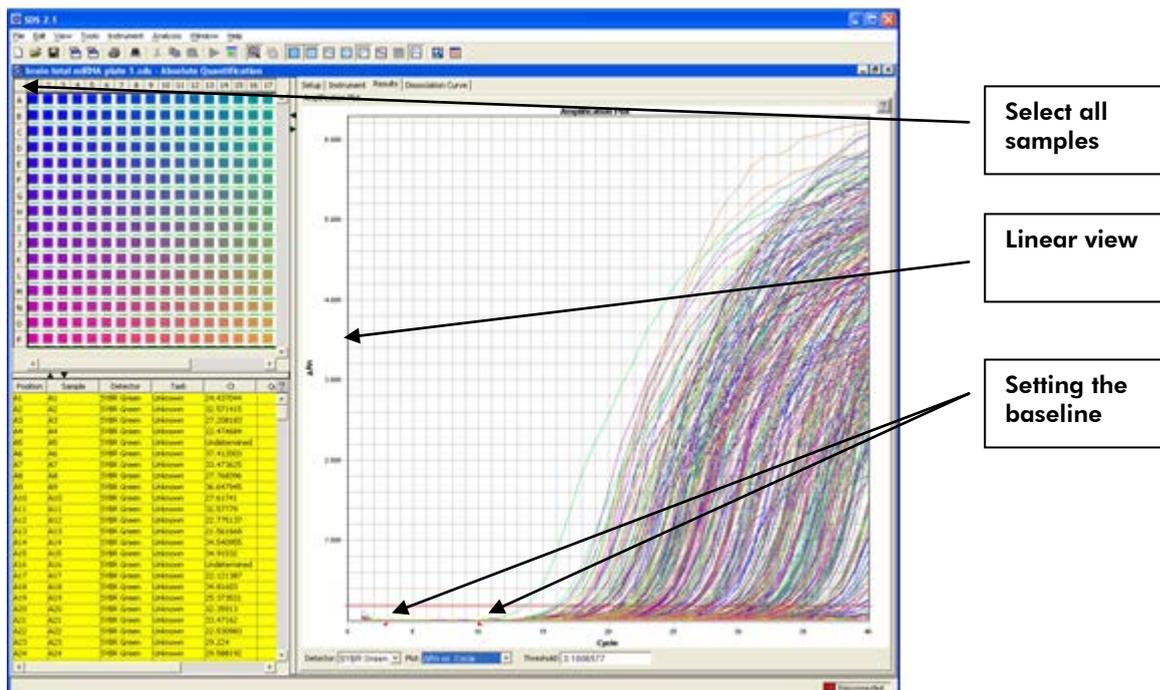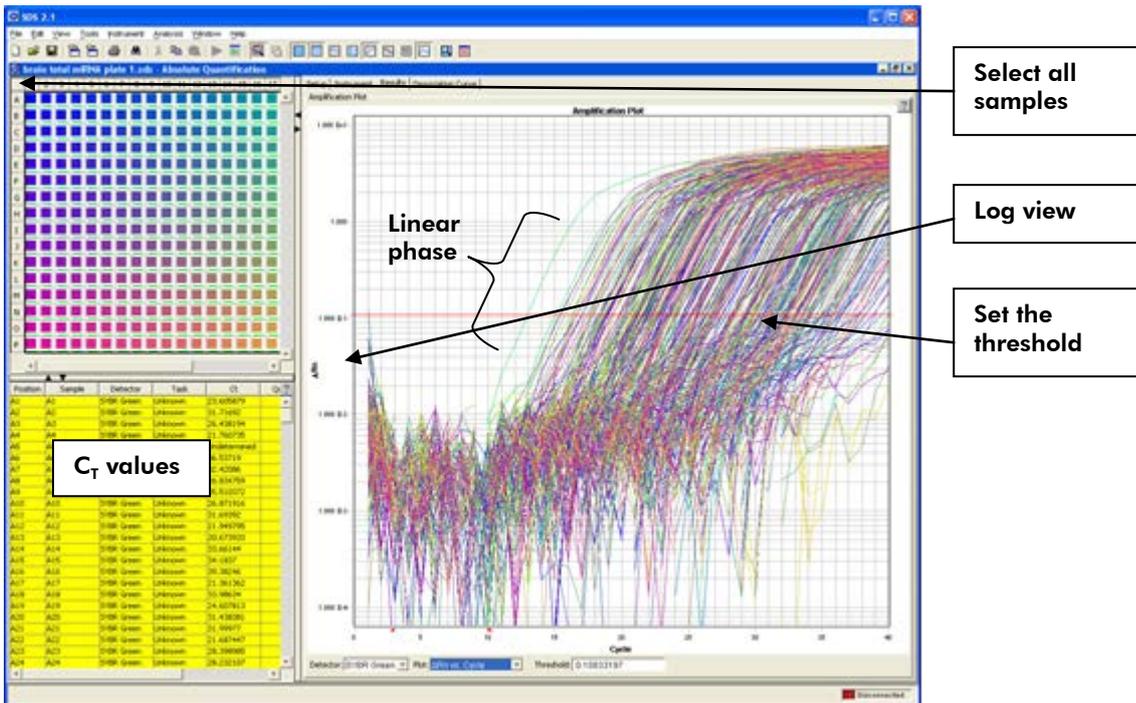


Figure 4. Setting the baseline.

**Figure 5. Setting the threshold.**

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