Protocol Sheet

Life Technologies® ViiA-7® (ViiA 7 Software v1.2) 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.
- Please make sure the real-time PCR instrument 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 ViiA 7 Software version on your instrument.
- Set up a PCR protocol template file on the LIFE TECHNOLOGIES ViiA 7 Real Time PCR System VIIA 7 software as follows.

Procedure

Creation of PCR protocol template

1. **Open the Life Technologies VIIA 7 Software on the desktop of the computer that is connected to the Life Technologies ViiA 7 Real Time PCR System system.**
2. **Select Experiment Set Up, or the New Experiment icon. A Setup page will appear. From left, the first tap is Experiment Properties.**
    - **How do you want to identify this experiment?**
        - **Input your desired name; data and time are default**
    - **Which block are you using to run the experiment?**
        - **384-well block**
        - **96-well block**
        - **Fast 96-well block**
    - **Which type of experiment do you want to set up?**

- **Standard Curve**

- **Which reagents do you want to use to detect the target sequence?**
  - **SYBR® Green Reagents**
- **What properties do you want for the instrument run?**
  - **Standard**
  - **Fast (if you choose Fast Mastermix)**

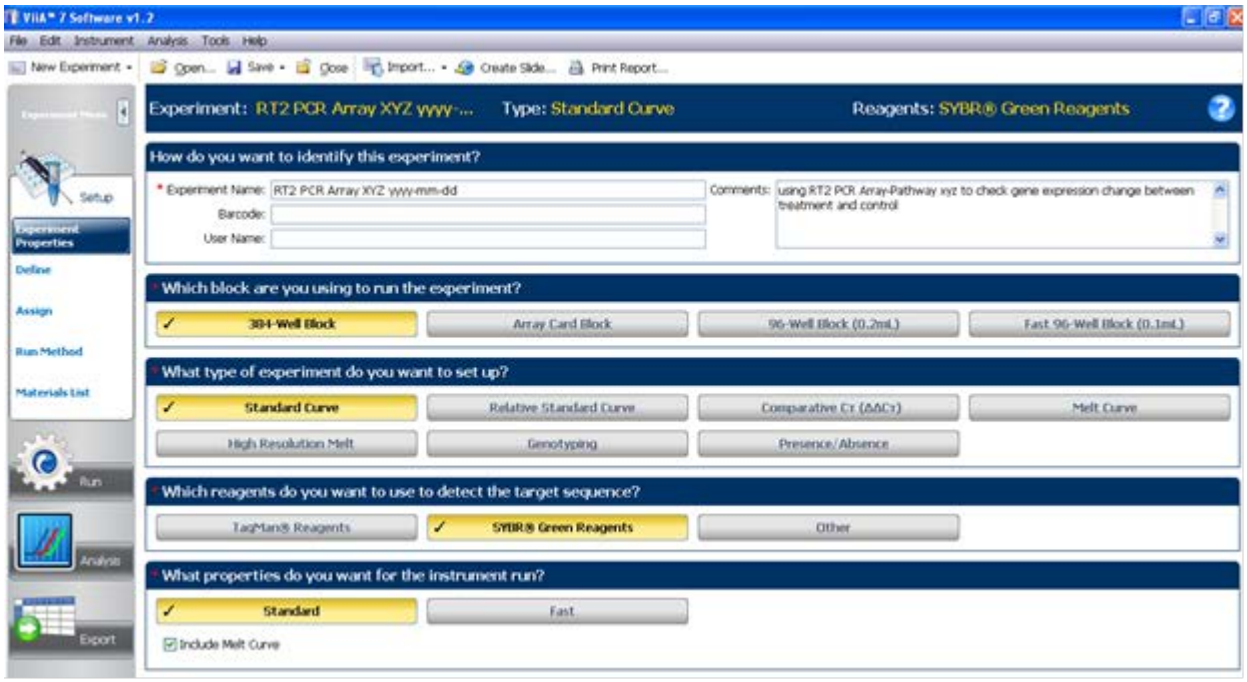


Figure 1. Experiment identification screen.

3. Select the second tab from the left, Define

- **Under Targets:**
  - **Leave Target Name as default Target 1, or enter a Target Name**
  - **Select Report, and choose SYBR**
  - **Select Quencher, and choose None**
  - **Select desired color (for example, green)**
  - **Do not modify Biological Replicate Groups**
  - **Samples: change sample names**
  - **For passive reference, select ROX**

4. Select the third tab from the left, Assign

- **Under Plate Layout, select all wells by clicking the upper left-hand corner**
- **Under Targets, select Target 1 by clicking the selection button on the left (if setting 96 or 384 targets, select a different target for each well)**
- **Under Samples, select all wells as Sample 1 by clicking selection button on the left**

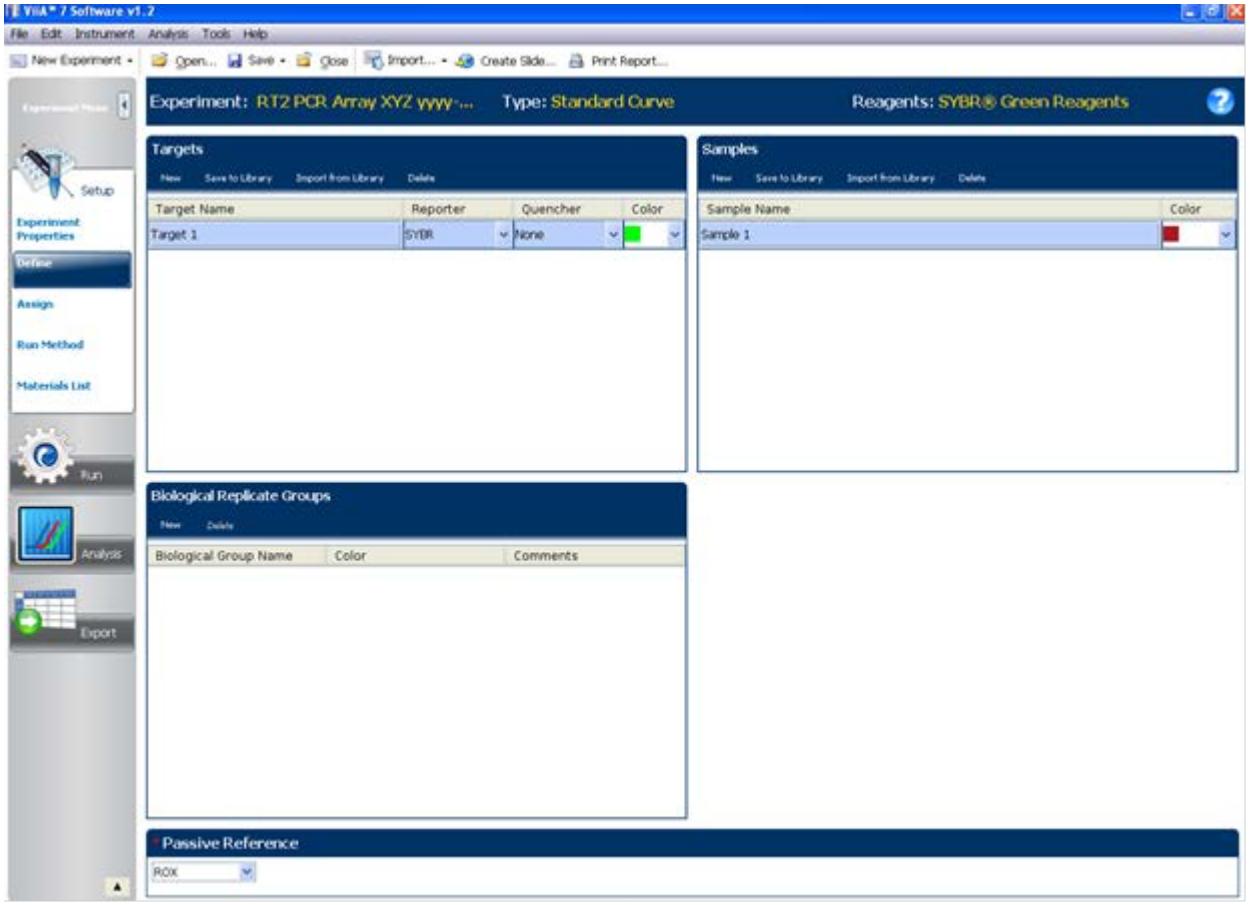


Figure 2. Targets screen.

5. Select the fourth tab from the left, Run Method

- **Under Run Method, change Reaction Volume per Well to 10 µl for a 384-well plate, or 25 µl for the 96-well plate**
- **Under Graphical View, Hold Stage, select 50.0C 02:00 Step 1, then click Delete Selected to remove this step. Use 95.0°C 10:00 as Hold Stage setting.**
- **Under Graphical View, PCR Stage, select Number of Cycles as 40. Make sure settings are:**
    - **95.0°C 00:15**
    - **60.0°C 01:00**
- **Under Graphical View, Melt Curve Stage, select Continuous with the default setting**

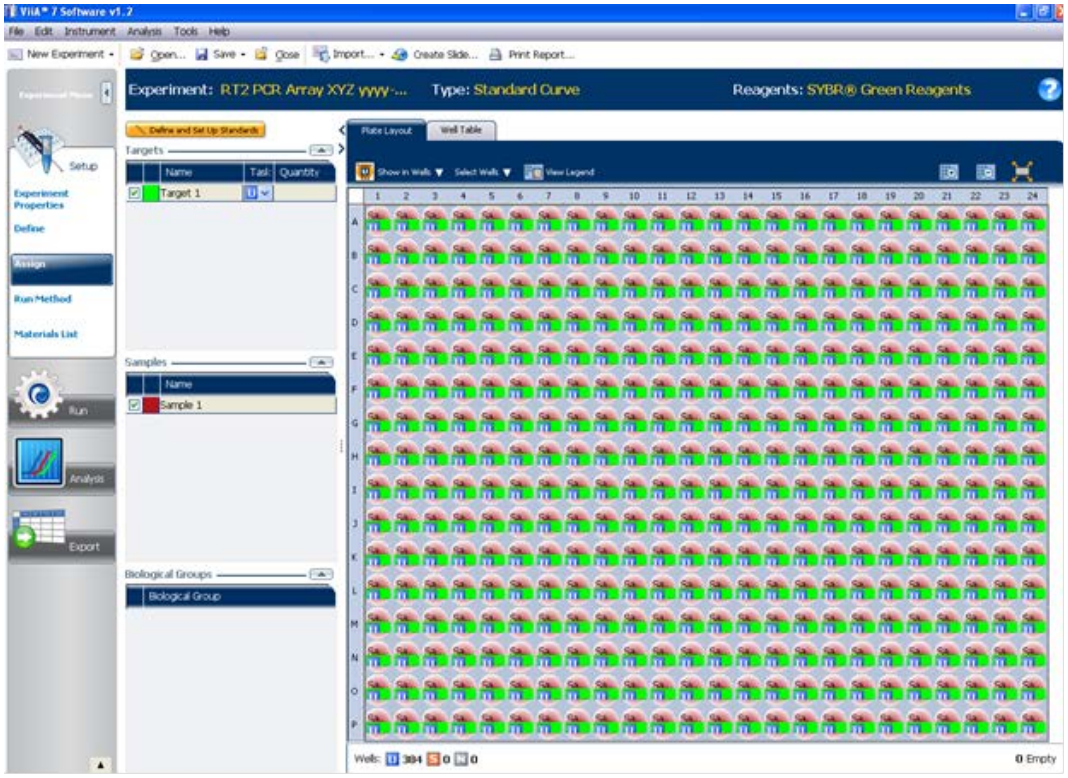


Figure 3. Plate layout.

6. Materials List does not need modification

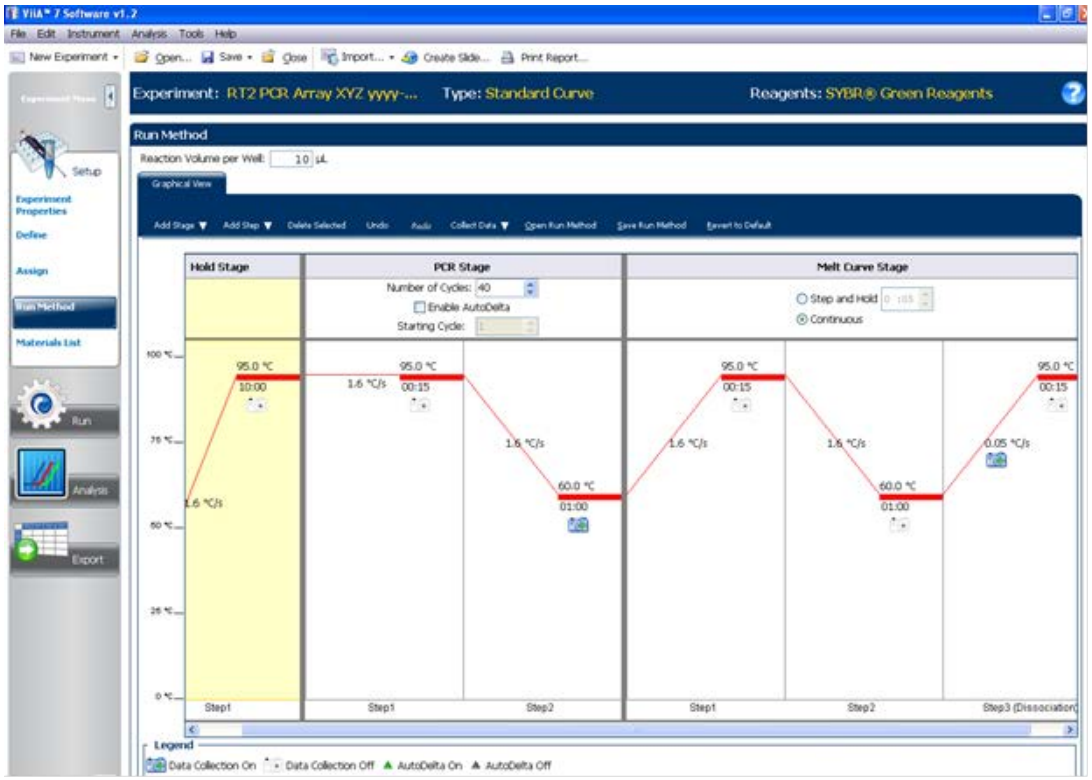


Figure 4. Graphical view screen.

7. **Under File in the top left corner, select Save as Template, then name your template and save**
8. **Alternatively, download the PCR protocol template file (RT² Profiler™ PCR Array Protocol Template-LIFE TECHNOLOGIES_ViiA7.sdt) from the QIAGEN-SABiosciences website http://www.SABiosciences.com/home.php.**

Performing real-time PCR detection

9. **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.**
10. **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.**
11. **Open the Life Technologies VIIA 7 Software**
12. **Select File ➤ New Experiment➤From Template. Browse to load the RT² Profiler PCR Array Protocol Template LIFE TECHNOLOGIES_ViiA7 file. This will load the previously saved setup to the new plate document.**
13. **Change the name of document under a new filename if you do not want to use the default date/time-based name.**
14. **Click 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.**

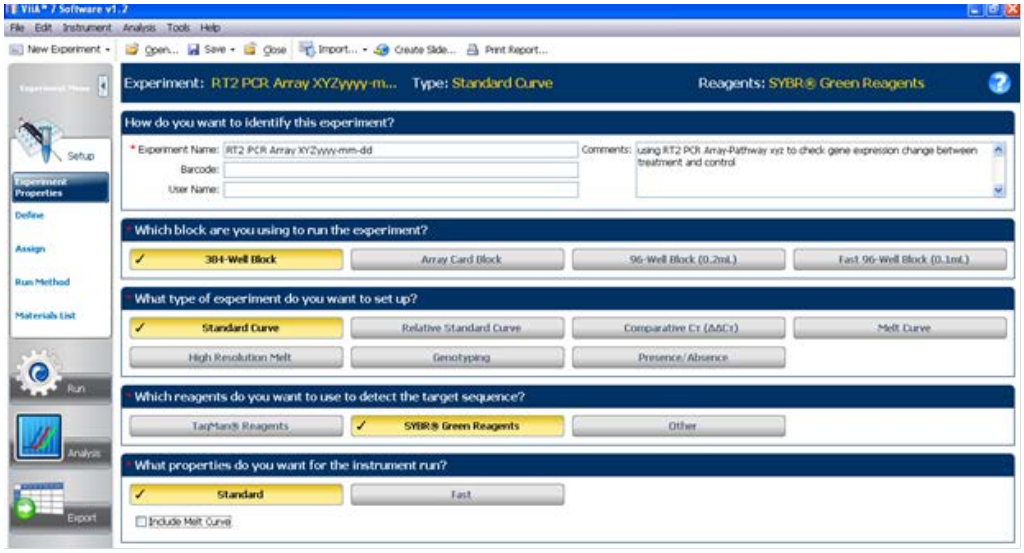


Figure 5. Setup screen.

15. **Click Next.**
16. **Click Start Run.**
17. **Enter name for run and click Save.**
18. **Rotor-Gene Q run will now commence.**

After the PCR run

19. **When the PCR run is complete, a small dialog box stating "The run completed successfully" will appear on the screen. Click OK to close the box.**
20. **For analyzing old data, first click Open.**
21. **Select Analysis ➤ Amplification Plot tab on the left (the Analysis Settings button is on the top right corner).**
22. **Click Analysis Settings button to get the Analysis Settings page.**
23. **Select Data Step, and select the default Stage 2, Step 2.**
24. **For Algorithm Settings, use the default Baseline Threshold.**
25. **Default Ct Settings: Change the default settings by clicking Edit Default Settings to create a new page. Select Threshold 0.02 (or the threshold selected based on the method described in step 29) and Automatic baseline. Then click Save Changes.**
26. **Under Select a Target, your target such as Target 1 (default) is selected with the current setting. If you have already set up your targets, you can go to the right to select all or each individual target that you wish to analyze.**

27. **Under Ct Settings for Target 1, please select Default settings if you already set the default setting for the RT^2 Profiler PCR Array. Otherwise, deselect the Default Setting, then input Threshold 0.02 (or the one that you selected based on the method described in step 29) and select Automatic Baseline for analysis. This will be reflected under the Select a Target tab.**

28. **Click Apply Analysis Settings on the bottom, and a Progress information bar will show the analysis progress.**

29. **If you want to set up the baseline manually and select the best fit threshold for your whole study, follow the procedures below to calculate the threshold cycle (Ct) for each well (See Figures 6 and 7):**

    Note: We highly recommend manually setting the Baseline and Threshold Value

    - **To define the Baseline, use the Linear View of the amplification plots. Double-click on the 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. Use the red sliding bars on the 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 the 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.**

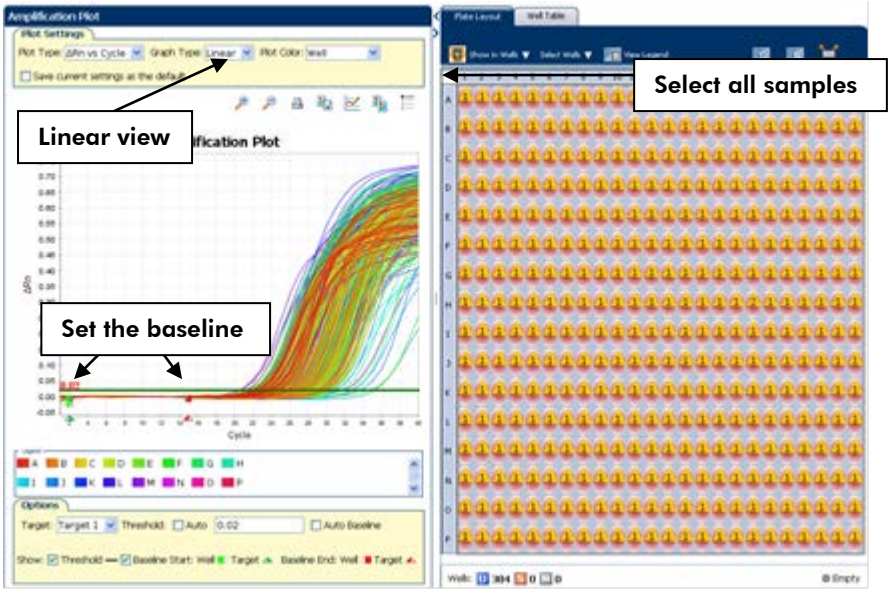


Figure 6. Setting the baseline.

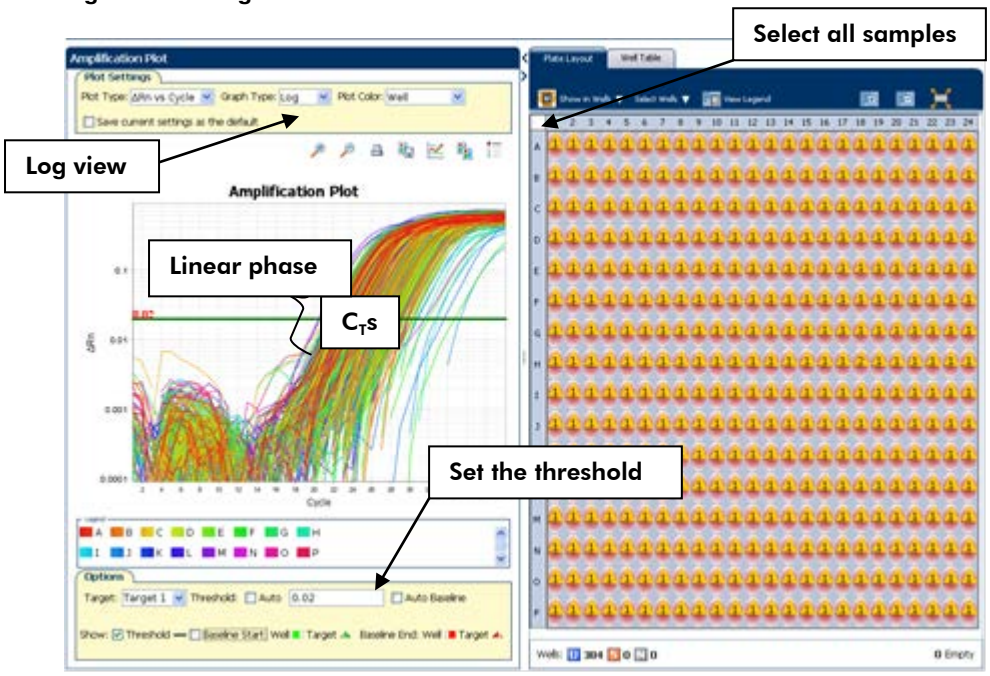


Figure 7. Setting the threshold.

- **The values for C_T will be displayed in the amplification plot if you choose C_T vs well.**
- **To export the result to an Excel spreadsheet, select Export➤Export**
  - **Default Format is ViiA7**
  - **Select one file for the Export Data To option**
  - **To see the result directly, select Open file(s) when export is complete**
  - **Export File Location: select desired folder on your computer**

- **Export File Name: input desired file name**
- **File Type: select *.xls**
- **Select Results tab (can select other information such as sample setup, Raw Data, Amplification and Multicomponent Data, if desired)**
- **Under Select Content, can select All Fields or select fields of interest, such as C_T and C_T Threshold and Tm1, Tm2.**
- **Click Start Export. To see the Excel file immediately, select Open files when export is complete.**

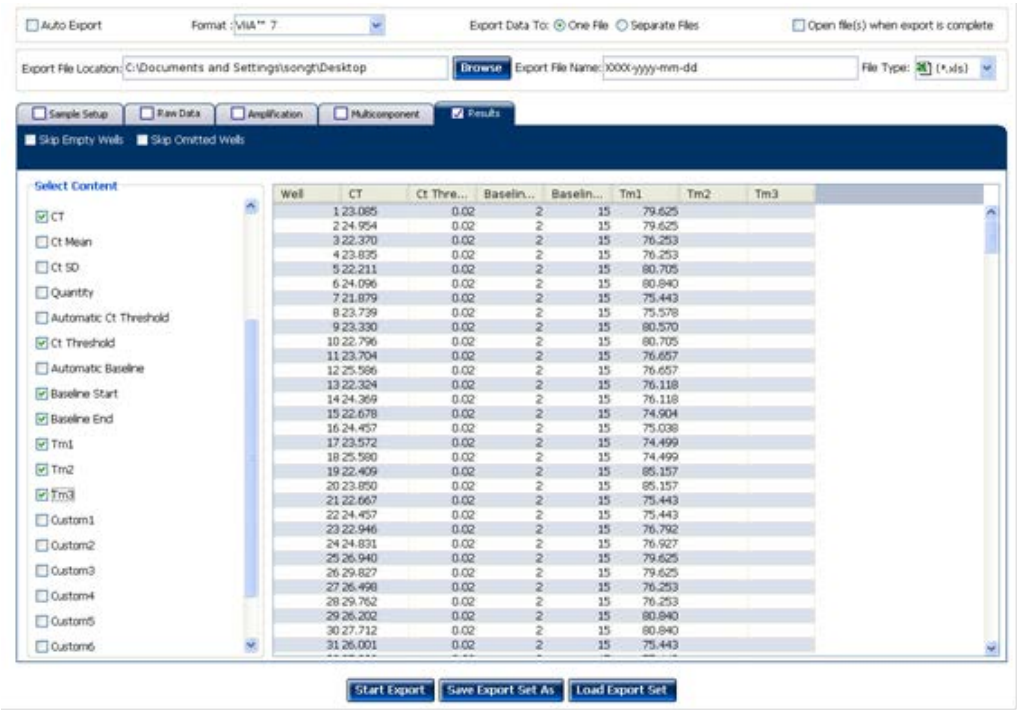
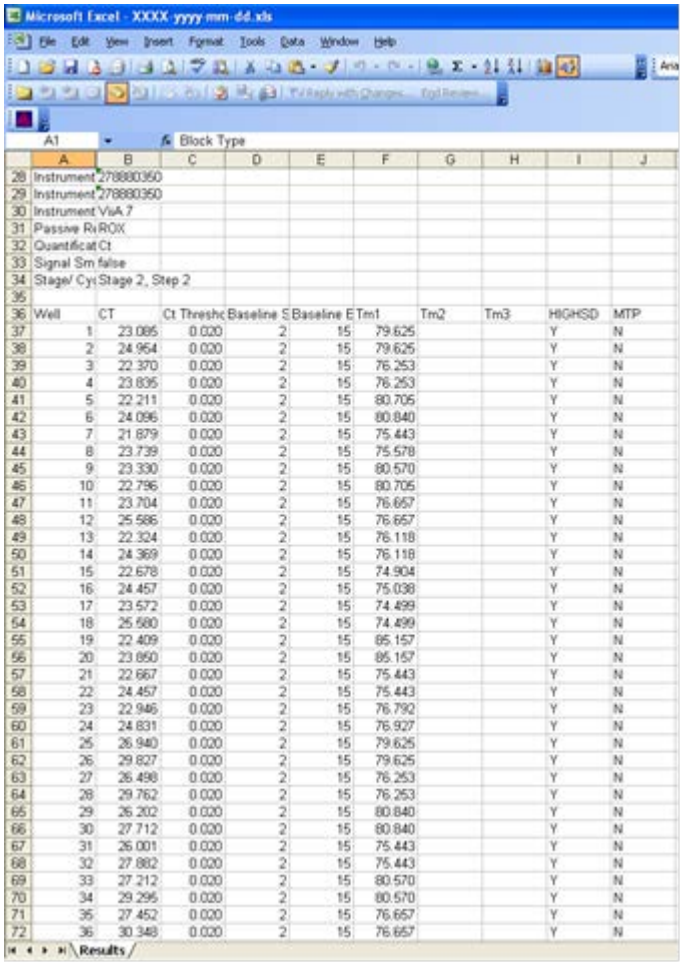


Figure 8. Export data screen.



Microsoft Excel - XXXX-yyyy mm-dd.xls

File Edit View Insert Format Tools Data Window Help

A1 Block Type

	A	B	C	D	E	F	G	H	I	J
28	Instrument	278880350								
29	Instrument	278880350								
30	Instrument	ViiA 7								
31	Passive Re	ROX								
32	Quantificat	Ct								
33	Signal Sm	false								
34	Stage/ Cyc	Stage 2, Step 2								
35										
36	Well	CT	Ct Threshc	Baseline S	Baseline E	Tm1	Tm2	Tm3	HIGHSD	MTP
37	1	23.086	0.020	2	15	79.625			Y	N
38	2	24.964	0.020	2	15	79.625			Y	N
39	3	22.370	0.020	2	15	76.253			Y	N
40	4	23.835	0.020	2	15	76.253			Y	N
41	5	22.211	0.020	2	15	80.705			Y	N
42	6	24.096	0.020	2	15	80.840			Y	N
43	7	21.879	0.020	2	15	75.443			Y	N
44	8	23.739	0.020	2	15	75.578			Y	N
45	9	23.330	0.020	2	15	80.570			Y	N
46	10	22.796	0.020	2	15	80.705			Y	N
47	11	23.704	0.020	2	15	76.657			Y	N
48	12	25.586	0.020	2	15	76.657			Y	N
49	13	22.324	0.020	2	15	76.118			Y	N
50	14	24.369	0.020	2	15	76.118			Y	N
51	15	22.678	0.020	2	15	74.904			Y	N
52	16	24.457	0.020	2	15	75.038			Y	N
53	17	23.572	0.020	2	15	74.499			Y	N
54	18	25.680	0.020	2	15	74.499			Y	N
55	19	22.409	0.020	2	15	85.157			Y	N
56	20	23.050	0.020	2	15	85.157			Y	N
57	21	22.667	0.020	2	15	75.443			Y	N
58	22	24.457	0.020	2	15	75.443			Y	N
59	23	22.946	0.020	2	15	76.792			Y	N
60	24	24.831	0.020	2	15	76.927			Y	N
61	25	26.940	0.020	2	15	79.625			Y	N
62	26	29.827	0.020	2	15	79.625			Y	N
63	27	26.498	0.020	2	15	76.253			Y	N
64	28	29.762	0.020	2	15	76.253			Y	N
65	29	26.202	0.020	2	15	80.840			Y	N
66	30	27.712	0.020	2	15	80.840			Y	N
67	31	26.001	0.020	2	15	75.443			Y	N
68	32	27.882	0.020	2	15	75.443			Y	N
69	33	27.212	0.020	2	15	80.570			Y	N
70	34	29.296	0.020	2	15	80.570			Y	N
71	35	27.452	0.020	2	15	76.657			Y	N
72	36	30.348	0.020	2	15	76.657			Y	N

Results

Figure 9. Exported C_T result in Excel file.

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

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature . Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx .

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