Protocol Sheet

Life Technologies® ViiA®7 (for ViiA7 Software version 1.2) instrument setup instructions for qBiomarker Copy Number PCR Arrays

Important points before starting

■ Please read the handbook supplied with the qBiomarker Copy Number PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

■ 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 ViiA 7 Software version on your instrument.

Procedure

Setting up a 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.

2. Select "Experiment Set Up," or the "New Experiment" icon; a setup page will appear. From left, the first tab is "Experiment Properties."

    ▪ "How do you want to identify this experiment?" ➤

        • Input your desired name; default is the date and time.

    ▪ "Which block are you using to run the experiment?" ➤

        • "384-Well Block" or

        • "96-Well Block" or

        • "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" or

        • "Fast" if Fast master mix is used
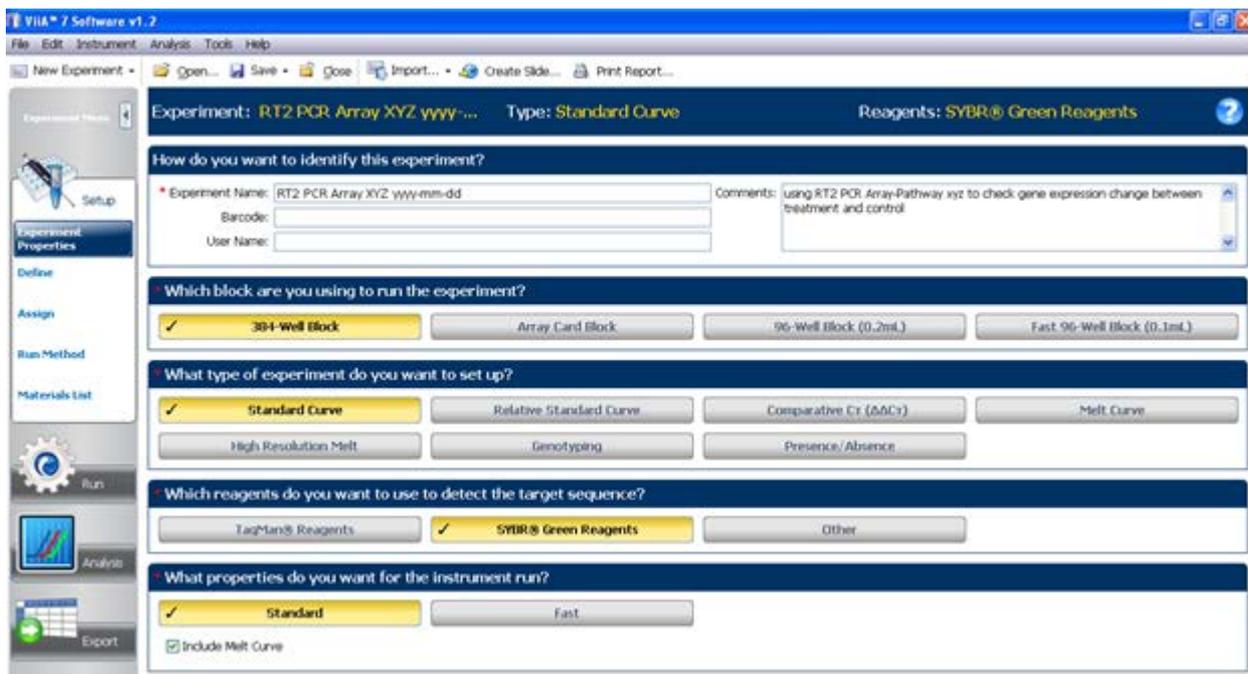
Figure 1. "Experiment Properties" screen.

3. Select the second tab from left, "Define."

  ▪ Under the "Targets" section:

     • Leave the "Target Name" as the default, "Target 1," or enter a target name.

     • Select "Reporter"; choose "SYBR."

     • Select "Quencher"; choose "None."

     • Select a desired color, such as green.

     • No need to modify "Biological Replicate Groups."

     • "Samples": change sample name, if desired.

     • "Passive Reference"; select "ROX."

4. Select the third tab from left, "Assign."

  ▪ Under the "Plate Layout," select all wells by clicking the upper-left corner.

  ▪ Under the "Targets," select "Target 1" by clicking the selection button on its left (if you set 96 or 384 targets, select a different target for each well).

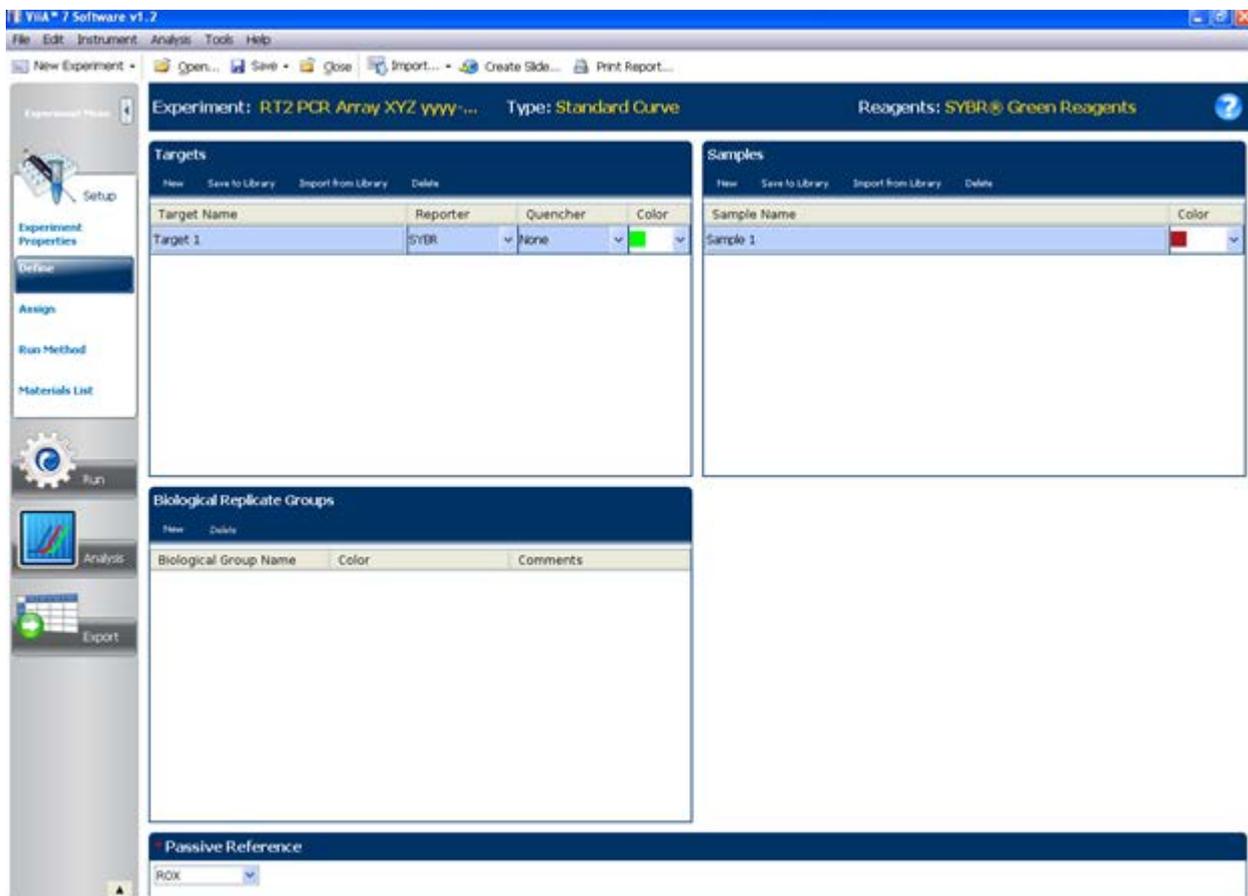 ▪ Under "Samples," select all wells as "Sample 1" by clicking the selection button on its left.

Figure 2. "Define" screen.

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

    ▪ Under "Run Method," change the "Reaction Volume per Well" to 10 μl for 384-well plate or 25 μl for 96-well plate.

    ▪ Under "Graphical View," "Hold Stage," select "50.0°C 02:00 Step 1," and then click "Delete Selected" to remove that step. Use "95.0°C 10:00" as "Hold Stage" setting.

    ▪ Under "Graphical View," "PCR Stage," select "Number of Cycles" as 40. Make sure the settings are:

    - 95.0°C 00:15
    - 60.0°C 01:00

    ▪ Under "Graphical View," "Melt Curve Stage," select "Continuous" with the default setting.

6. No adjustments are needed for "Materials List."

7. Under "File" in the top left corner, select "Save as Template," then name your template and save.
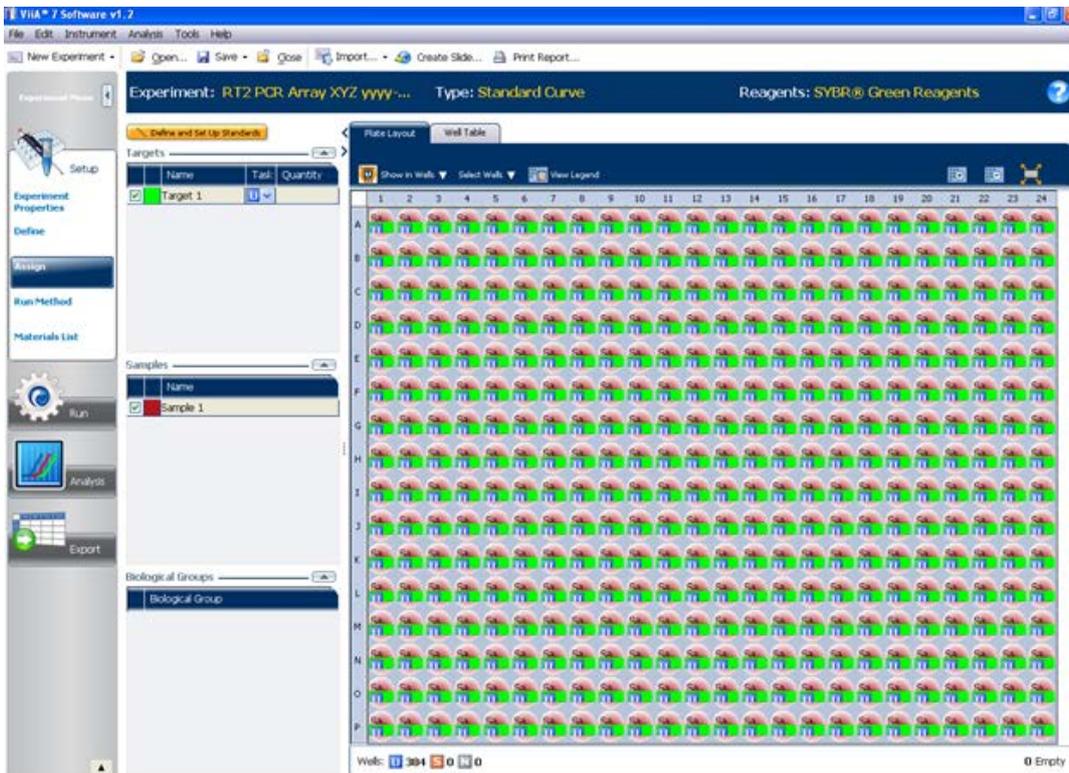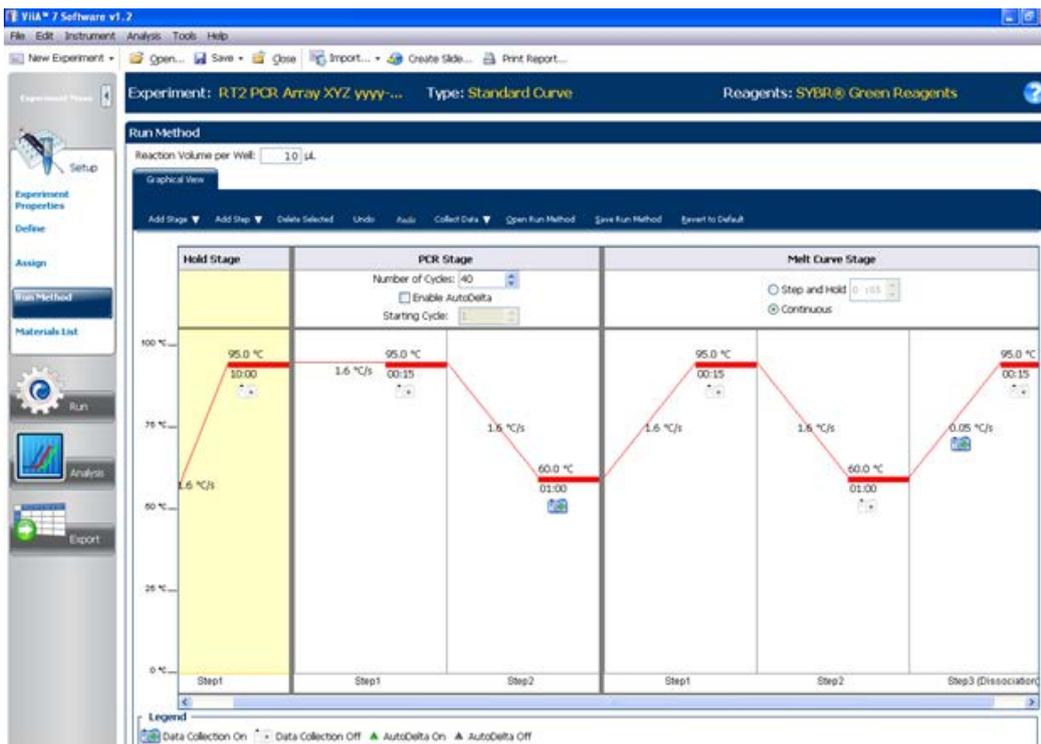
Figure 3. "Assign" tab.

Figure 4. "Run Method" tab.

Performing real-time PCR detection

1. 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.

2. 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.

3. Open the Life Technologies ViiA7 Software.

4. Select "File" ➤ "New Experiment" ➤ "From Template... Browse" to load the template file saved in Step 7 above. This will load the previously saved setup to the new plate document.

5. Change the name of document under a new filename if you do not want to use the default date and time-based name.

6. Click "Run" to begin the PCR run. Wait 30 seconds to 1 min for the initial priming, and then the run should start. The estimated run time will then appear on the screen.
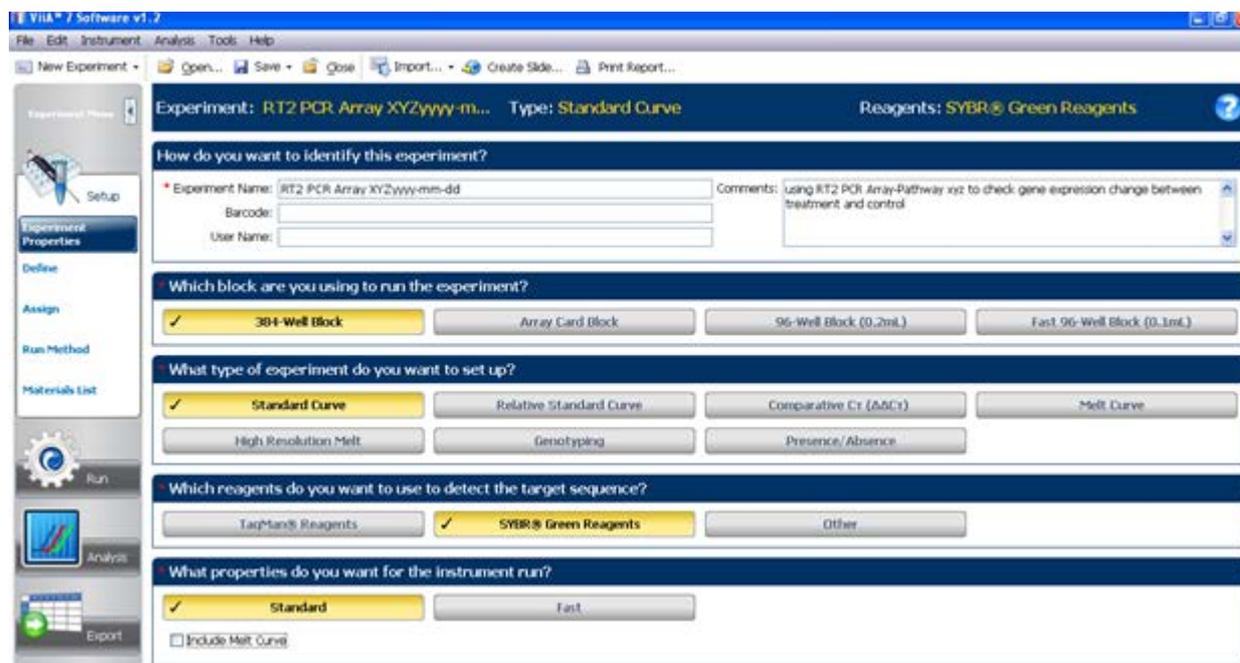

Figure 5. "Experiment Properties" screen.

After the PCR run

1. 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.

2. If you want to perform analysis for previously acquired data, click "Open" at the top of the page.

3. Select "Analysis ➤ Amplification Plot" tab on the left; the "Analysis Settings" button is on the top right corner.

4. Click the "Analysis Settings" button to open the "Analysis Settings" page.

5. Select "Data Step," using the default "Stage 2, Step 2."

6. Select "Algorithm Settings," using the default Baseline Threshold.

7. Default C_T settings: Change the default setting by clicking "Edit Default Settings" to open a new page. Select "Threshold 0.02" (or the threshold selected based on the method described in step 24) and "Automatic Baseline." Then click "Save Changes."

8. Under "Select a target," the target such as "Target 1" (default) is selected with the current setting. If 96 targets have already been set up, for example, select all of them together or each individual target that you wish to analyze.

9. Under "C_T Settings for Target 1," please select "Default Settings" if you have 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 24) and select "Automatic Baseline" for analysis. This will be reflected under the "Select a Target" tab.

10. Click "Apply Analysis Settings" on the bottom, and a "Progress" information bar will show the progress of the analysis.

11. 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 (C_T) for each well (See figures 4 and 5):

Note: We highly recommend manually setting the "Baseline" and "Threshold" values.

- 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 the 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 the 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 amplification plot if you choose "C_T vs well."
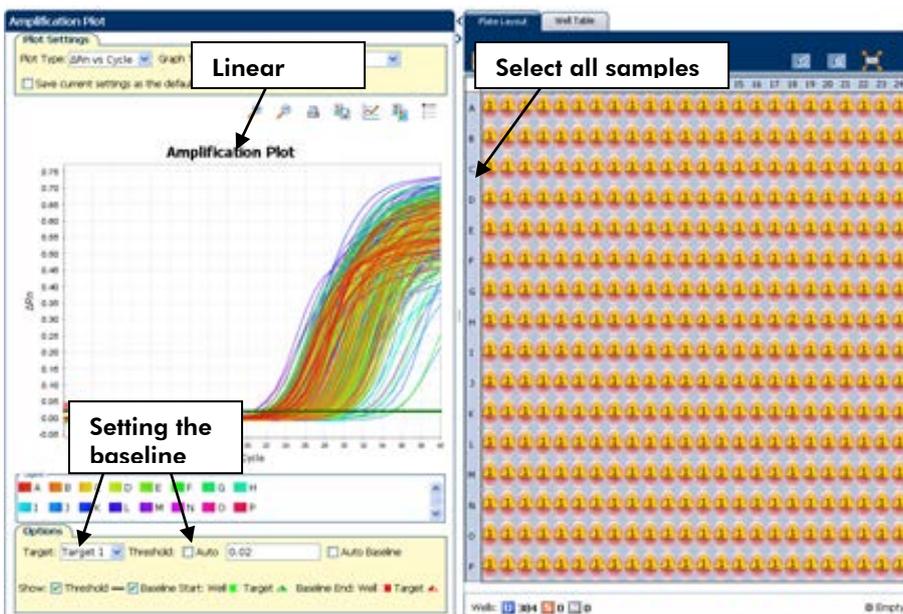
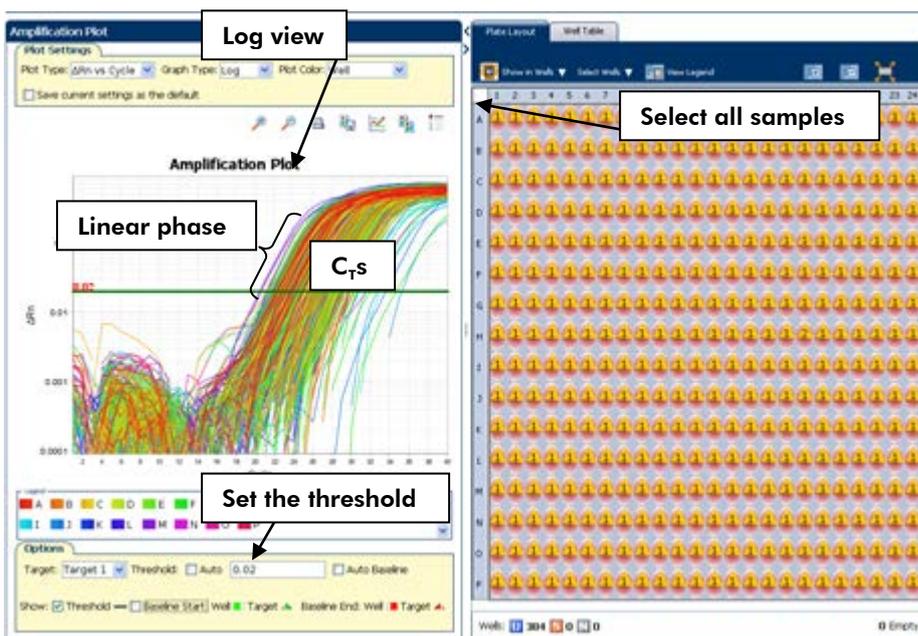**Figure 6. Setting the baseline.**

Figure 7. Setting the threshold.

- To export the result to an Excel® spreadsheet, select "Export ➤ Export."
    - Format: default is ViiA7
    - For "Export data to", choose "One File."
    - To see the file immediately, select "Open file(s) when export is complete."
    - "Export File Location": Browse your computer to select the desired folder.
    - "Export File Name": please input desired file name.
    - "File Type": select *.xls.
    - Select "Results" tab (you also can select other information such as sample setup, raw data, amplification, and multicomponent data if desired).
    - Under "Select Content," choose "All Fields" or select an individual field, such as "C_T and C_T Threshold" and "Tm1, Tm2."
    - Click "Start Export." The result will appear in an Excel file immediately if "Open files when export is complete" was selected.

The qBiomarker Copy Number PCR Arrays are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

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