Quantifiler™ Human DNA Quantification Kits on the ABI PRISM® 7000 Sequence Detection System

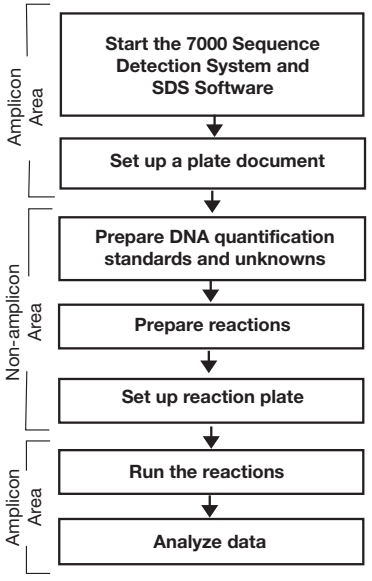
For chemical and instrument safety guidelines, refer to the "Safety" section in the preface of the *Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual* (PN 4344790). Refer to this user manual for more detailed information on the procedures.

Product Overview

The *Quantifiler™ Human DNA Quantification Kit* and the *Quantifiler™ Y Human Male DNA Quantification Kit* are designed to quantify the total amount of amplifiable human (and higher primate) DNA or human male DNA in a sample.

This quick reference card covers setting up the plate document, running PCR, and viewing results. Refer to the Quantifiler kits user's manual or the *Quantifiler™ Human DNA Quantification Kits PCR Amplification Quick Reference Card - Non-Amplicon Area Only*, PN 4352952, for preparation of the DNA standards and sample reactions.

Workflow



Materials

Material	Source
Quantifiler Human DNA Quantification Kit	Applied Biosystems (PN 4343895)
Quantifiler Y Human Male DNA Quantification Kit	Applied Biosystems (PN 4343906)
Glycogen, 20 mg (1 mL)	Roche Applied Science (PN 901 393)
Equipment	**Source**
ABI PRISM® 7000 Sequence Detection System	See your Applied Biosystems Sales Representative
96-Well Optical Reaction Plates	Applied Biosystems (PN 4306737)
Optical Adhesive Covers Starter Kit	Applied Biosystems (PN 4313663)
Optical Tubes	Applied Biosystems (PN 4316567)
Optical Caps	Applied Biosystems (PN 4323032)

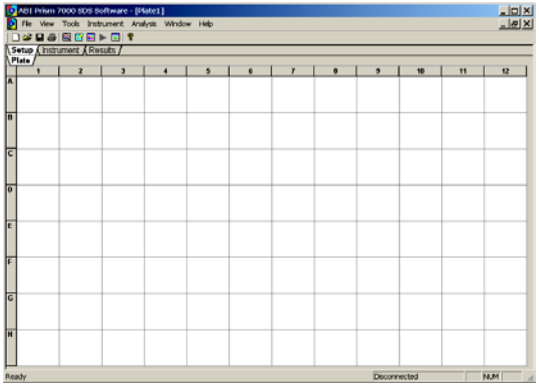
Figure 1 The *Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit.*

Starting the 7000 Sequence Detection System (SDS)

1. Power on the computer.
2. Type your user name and password.

   IMPORTANT! Wait for the computer to finish starting up before powering on the 7000 SDS instrument.
3. Power on the instrument.
4. Select **Start>ABI PRISM® 7000>ABI PRISM® 7000 SDS Software**.

A blank plate document is displayed.



Setting Up a Plate Document

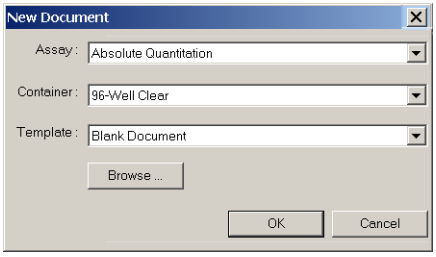
Set up the plate document so that it corresponds exactly to the arrangement of the standards and unknown samples in the wells of the reaction plate. See the example provided below in Table 1.

Table 1 Example plate document

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Std 1	Std 1	Std 2	Std 2	Std 3	Std 3	Std 4	Std 4	Std 5	Std 5	Std 6	Std 6	Reactions using the Quantifiler Human Kit
B	Std 7	Std 7	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	
C	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	
D	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	
E	Std 1	Std 1	Std 2	Std 2	Std 3	Std 3	Std 4	Std 4	Std 5	Std 5	Std 6	Std 6	Reactions using the Quantifiler Y Human Male Kit
F	Std 7	Std 7	Std 8	Std 8	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	
G	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	
H	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	UNKN	

Creating a Blank Plate Document

1. In the SDS software, select **File > New**.



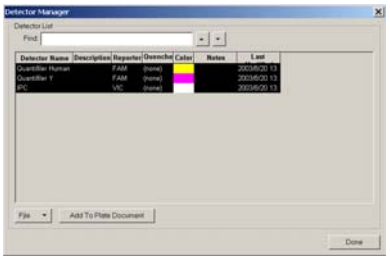
2. Click **OK**.

Creating Detectors (for first time use of Quantifiler kits)

Before you set up the plate document, create detectors in the SDS software for running Quantifiler kit assays. After the detectors are created, you do not need to create detectors for subsequent runs of Quantifiler kits. See the *Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual* for the procedure.

Adding Detectors to the Plate Document

1. Select **Tools > Detector Manager**.
2. Select the **Quantifiler Human**, **Quantifiler Y**, and the **IPC** detectors by clicking them while pressing the Ctrl key.



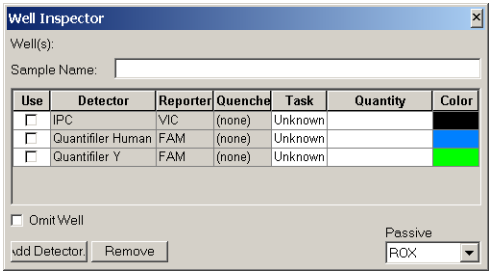
3. Click **Add To Plate Document**.
4. Click **Done**.

Applying Detectors for Standards

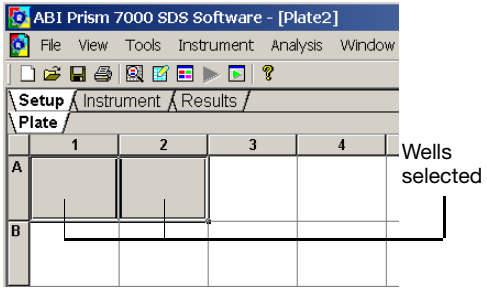
Note: Repeat until you complete applying detector tasks, quantities, and sample names for all quantification standards.

IMPORTANT! Set up detectors for each quantity and for each kit separately.

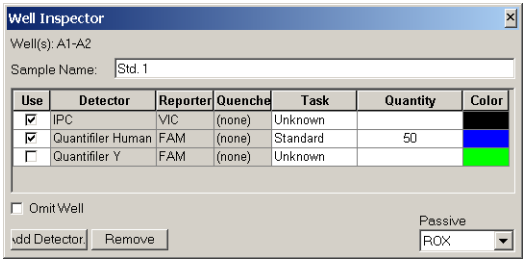
1. Select **View > Well Inspector**.



2. On the Plate tab, select wells that correspond to a specific quantification standard for one kit.



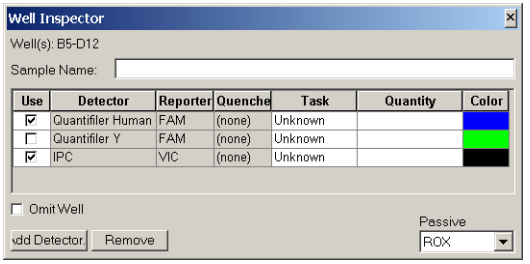
3. With the wells selected, go to the **Well Inspector** and select the **Use** boxes for the applicable detectors:
   - IPC
   - Quantifiler Human or Quantifiler Y
4. For Quantifiler Human or Quantifiler Y detector:
   a. Click **Unknown** in the Task column, then select **Standard** from the drop-down list.
   b. Select the Quantity field for the appropriate detector and enter the quantity of DNA in the well.
5. Enter the Sample Name.



Applying Detectors for Unknown Samples

IMPORTANT! If you run reactions for the Quantifiler Human kit and the Quantifiler Y kit on the same plate, apply detectors for unknown samples for each kit separately.

1. On the Plate tab, select the wells that correspond to all unknown samples for one Quantifiler kit.
2. With the well(s) selected, select **View > Well Inspector** and check the **Use** boxes for the applicable detectors:
   - Quantifiler Human or Quantifiler Y
   - IPC

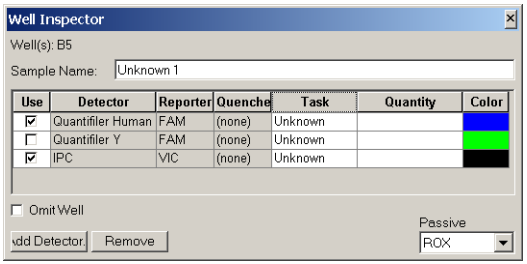


Note: Make sure **ROX** is selected.

3. If you are running both kits on the reaction plate, repeat the previous steps 1 and 2 for unknown samples for the other kit.
4. Select **View > Well Inspector** to close the Well Inspector.

Adding Sample Names for Unknown Samples

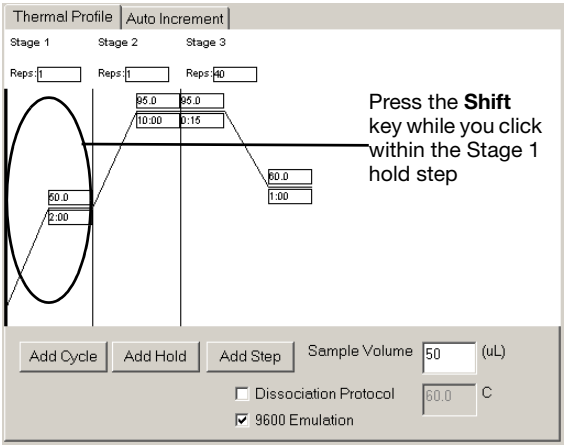
1. On the Plate tab, select one well containing an unknown sample.
2. With the well selected, select **View > Well Inspector** and enter the sample name.



Note: The SDS software will automatically group and analyze all replicate samples with the same name.

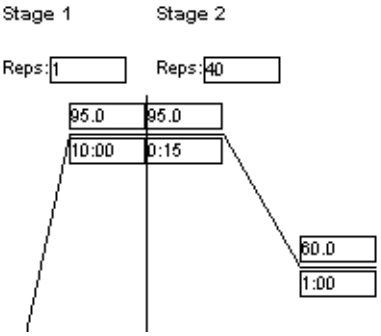
Setting Thermal Cycler Conditions

1. In the plate document, select the **Instrument** tab.
2. Press the **Shift** key and click within the Stage 1 hold step (50 °C for 2 minutes) to select it.

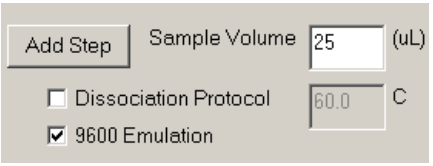


3. After the hold step is selected, press the **Delete** key.

4. Make sure the thermal profile appears as follows:



5. Change the Sample Volume to 25 (µL) and make sure that the 9600 Emulation box is selected.



Saving the Plate Document

1. Select **File > Save**.
2. Select the location for the plate document.
3. Enter a file name.
4. For Save as type, select **SDS Documents (*.sds)**.
5. Click **Save**.

PCR Amplification

Preparing Standards and Reactions

See the *Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual* or the *Quantifiler™ Human DNA Quantification Kits PCR Amplification Non-Amplicon Area - Quick Reference Card* for information about reaction preparation.

Running Reactions on the 7000 SDS Instrument

Before you run the reactions, make sure that you have powered on the computer, software, and instrument, and set up a plate document for the run.

1. Open the instrument door.

2. Position the plate in the instrument thermal block so that:
   - Well A1 is in the upper-left corner.
   - The notched corner of the plate is in the upper-right corner.
   - The plate is sitting down in the wells.
3. Gently push then release the carriage to unlatch it. The carriage automatically slides forward into position over the sample plate.

4. After the door moves to the front, pull the handle down into place to close the cover.

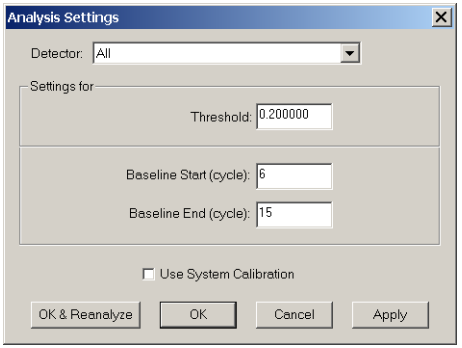
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block can be as high as 100 °C. Before performing the procedure, keep hands away until the heated cover and sample block reach room temperature.

5. In the SDS software, open the plate document that you set up for the run.
6. Select the **Instrument** tab, then click **Start**.

Analyzing the Plate Document

For detailed information about viewing and interpreting results, see the ***Quantifiler™ Kits User's Manual***.

1. Open the plate document to analyze.
2. Verify the analysis settings:
   a. Select **Analysis > Analysis Settings**.
   b. Verify that the settings are as shown below, then click **OK**.



IMPORTANT! If the analysis settings differ from those shown here, change them to match the settings before clicking OK.

3. Select **Analysis > Analyze**.

Viewing Results

For more information about viewing and interpreting results, see the *Quantifiler™ Kits User's Manual*.

Viewing the Standard Curve

1. In the Results tab, select the **Standard Curve** tab.
2. In the Detector drop-down list, select the detector that corresponds to the kit that you are using:
   - Quantifiler Human or
   - Quantifiler Y
3. View the C_T values for the quantification standard reactions and the calculated regression line, slope, y-intercept, and R^2 values:

   A slope close to –3.2 indicates optimal, 100% PCR amplification efficiency.

Kit	Typical Slope (range)	Average Slope
Quantifiler Human	–3.2 ±0.3	–3.1
Quantifiler Y	–3.3 ±0.3	–3.3

View the Amplification Plot

1. In the Results tab, select the **Amplification Plot** tab.
2. In the Detector drop-down list, select a detector:
   - Quantifiler Human or Quantifiler Y
   - IPC
3. Select the appropriate samples in the table below the amplification plot.
4. Make sure the Threshold is set to **0.20**, the default setting.

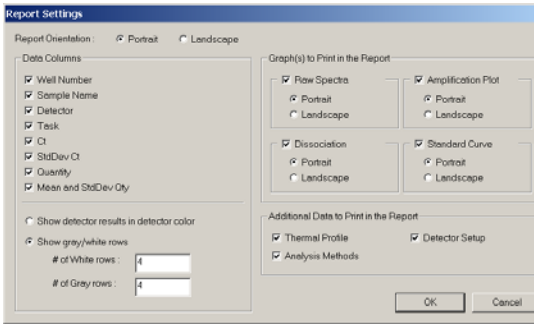
View the Report

1. In the analyzed plate document, select the Results tab, then select the Report tab.
2. Select the reactions in the 96-well plate representation below the report to display the results in the report.
3. View the **Qty** column to determine the quantity of DNA in each sample.

   Note: Quantities are calculated only if quantification standards were run and set up correctly in the software. Otherwise, only C_T values are shown.

Print or Export the Report

1. In the Report tab of the Results window, select **Tools > Report Settings**, then set up how the report is printed and exported.



2. Select **File > Print** to print the report.
3. Select **File > Export** to export the report as tab-delimited text.

Printed in USA,06/2004