

User Bulletin

Applied Biosystems 7900HT Fast Real-Time PCR System

October 3, 2005

SUBJECT: Performing Fast Gene Quantitation with 384-Well Plates

In This User Bulletin This user bulletin describes procedures for performing Fast gene quantitation with 384-well plates.

System Overview Fast gene quantitation involves running TaqMan® Gene Expression Assays and other quantitation assays on the Applied Biosystems 7900HT Fast Real-Time PCR System with 384-Well Block Module in less than 55 minutes.

- System Requirements**
- 7900HT Fast System with the following hardware and software:
 - 384-Well Block Module (thermal cycler block)
 - 384-Well Plate Adapter
 - Upgraded 48V-650 W power supply
 - Sequence Detection Systems Software v2.2.1 or later
 - Consumables:
 - Sequence Detection Systems 384-Well Spectral Calibration Kit (PN 4323977)
 - MicroAmp® Optical 384-Well Reaction Plate with Barcode (PN 4309849)
 - TaqMan® Fast Universal PCR Master Mix (2X), No AmpErase® UNG (PN 4352042)

- Before You Begin** Before you perform Fast gene quantitation, make sure that:
- You are familiar with the safety guidelines in the "Safety and EMC Compliance" section of the *Applied Biosystems 7900HT Fast Real-Time PCR System Site Preparation Guide* (PN 4351923)
 - SDS software v2.2.1 or later is installed.
 - The 384-Well Block and the 384-Well Plate Adapter are installed.
 - A background run and pure dye run have been performed and instrument performance has been verified within the last 6 months. For more information, refer to the *SDS Online Help* and the *Applied Biosystems 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide* (PN 4365542).

Singleplex vs. Multiplex Applications This user bulletin describes procedures for running singleplex applications. If you are performing multiplex applications (more than one target is amplified in a single tube), refer to [“Multiplex Applications” on page 10](#) for recommendations on how to optimize your assay performance.

**Related Products
for Fast Gene
Quantitation**

Product	Applied Biosystems Part Number
Applied Biosystems 7900HT Fast Real-Time PCR System with 384-Well Block Module	4329001
7900HT System Fast Service Upgrade	435142
7900HT System Fast Service Upgrade with Computer	4362078
Sequence Detection Systems 384-Well Spectral Calibration Kit	4323977
MicroAmp® Optical 384-Well Reaction Plate with Barcode	4309849
MicroAmp® Optical Adhesive Film, quantity 100 MicroAmp® Optical Adhesive Film, quantity 25	4311971 4360954
Sequence Detection Systems Software v2.2.1 or later	4361171
TaqMan® Fast Reagent Starter Kit	4352407
TaqMan® Fast Universal PCR Master Mix (2X), No AmpErase® UNG	4352042
TaqMan® Gene Expression Assays	4331182

**Related
Documents**

Document	Applied Biosystems Part Number
<i>Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i>	4364014
<i>Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide</i>	4364016
<i>Applied Biosystems 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide</i>	4365542
<i>Applied Biosystems Sequence Detection Systems Software version 2.3 Online Help (SDS Online Help)</i>	N/A
<i>Applied Biosystems 7900HT Fast Real-Time PCR System Site Preparation Guide</i>	4351923
<i>Real-Time PCR Systems Chemistry Guide</i>	4348358
<i>TaqMan® Fast Universal PCR Master Mix (2X) Protocol</i>	4351891
<i>TaqMan® Gene Expression Assays Protocol</i>	4364226

Performing 384-Well Fast Gene Quantitation

Overview Performing Fast gene quantitation involves:

- [Starting the Software](#) (page 3)
- [Creating and Setting Up a New Plate Document](#) (page 4)
- [Preparing Plates](#) (page 7)
- [Running Plates](#) (page 9)
- [Analyzing Results](#) (page 10)

Running Assays Using Fast Thermal Cycling Conditions

Run assays using Fast thermal cycling conditions.

- The performance of Fast thermal cycling and the TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG, has been verified for quantitative applications only and not for endpoint applications, such as allelic discrimination.
- The performance of Applied Biosystems TaqMan Gene Expression Assays and Custom TaqMan Gene Expression Assays has been verified using modified Fast thermal cycling conditions and the TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG.
- It is expected that the vast majority of custom 5' nuclease quantitation assays designed with the Applied Biosystems Assay Design Guidelines will provide comparable performance when run using the default Fast thermal cycling conditions and the TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG (as compared to running the standard thermal cycling conditions and the TaqMan® 2X Universal PCR Master Mix).
- When performing multiplex applications, you may need to perform some assay reoptimization. Before performing any multiplex applications, see the *Real-Time PCR Systems Chemistry Guide* (PN 4348358).

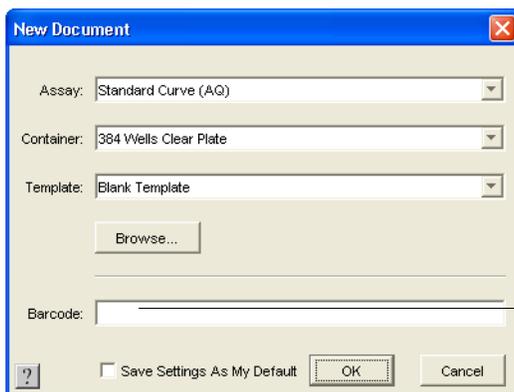
Starting the Software

Double-click  (SDS v2.2.1 or later) on the computer desktop.

At startup, the software establishes communication with the 7900HT instrument. If the connection is successful, the software displays  Connected to 'PlateName' in the status bar when a plate document is open.

Creating and Setting Up a New Plate Document

1. Create a plate document for 384-well fast gene quantitation:
 - a. In the SDS software, click  (or select File > New). Note that you can also create a new plate document using the New Plate Wizard, see:
 - *Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* (PN 4364014) or
 - *Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide* (PN 4364016).
 - b. Complete the New Document dialog box using the following settings, then click **OK**.



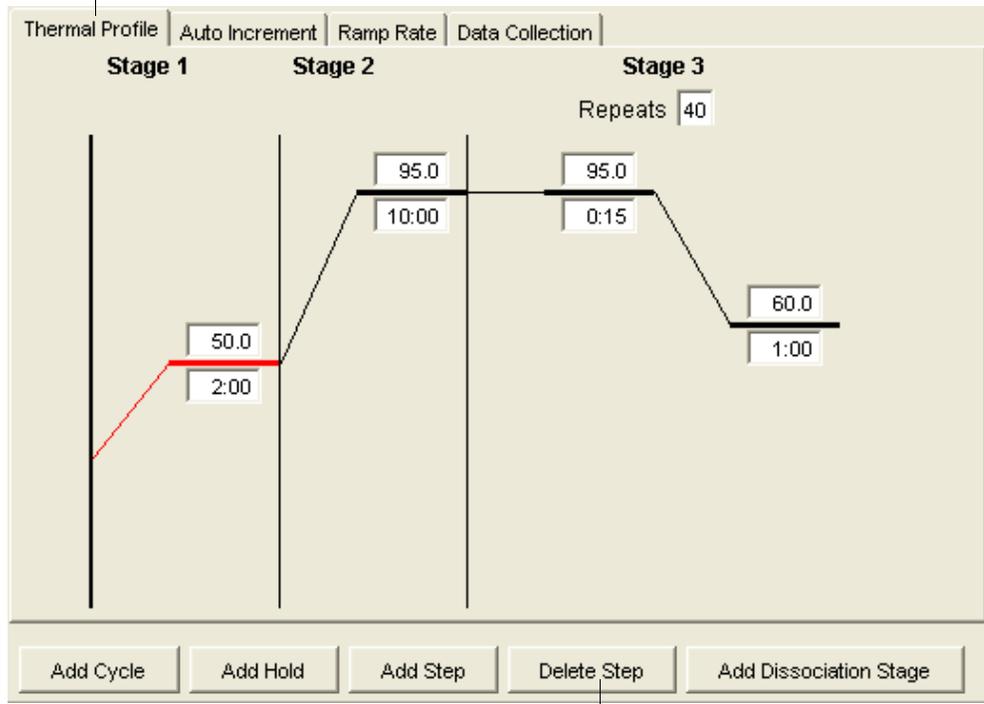
(Optional) Click the Barcode field, then scan or type the bar code.

2. Select the **Instrument** tab, then verify that Standard mode is selected and the Sample Volume is 20 μL .



- In the Thermal Profile tab, select **Stage 1** by clicking into (or near) the field boxes under that stage. Note that the line will turn red indicating that the stage is selected for editing. Then click **Delete Step**.

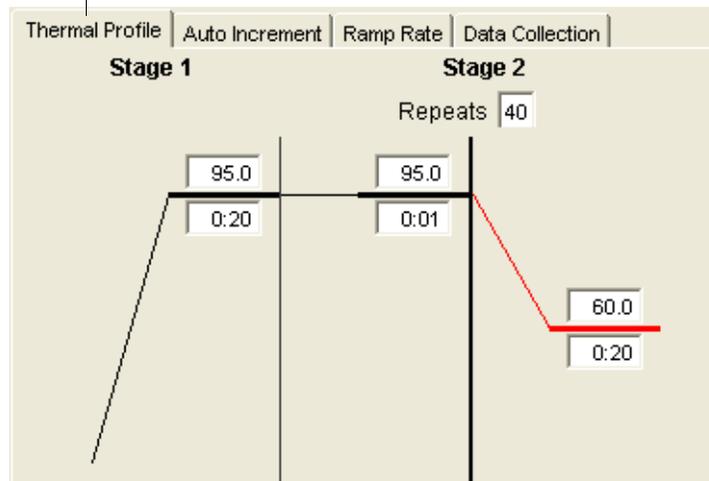
Thermal Profile tab



Delete Step

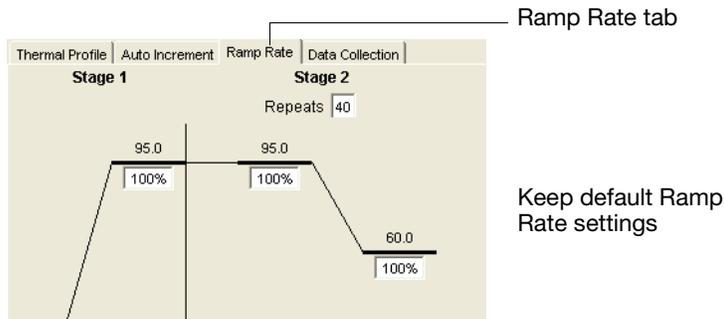
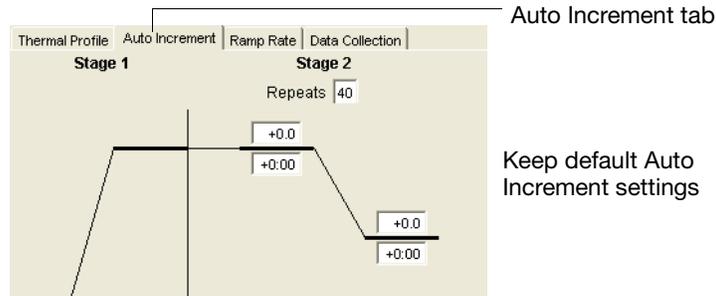
- Modify the remaining default thermal profile settings as shown below.

Thermal Profile tab

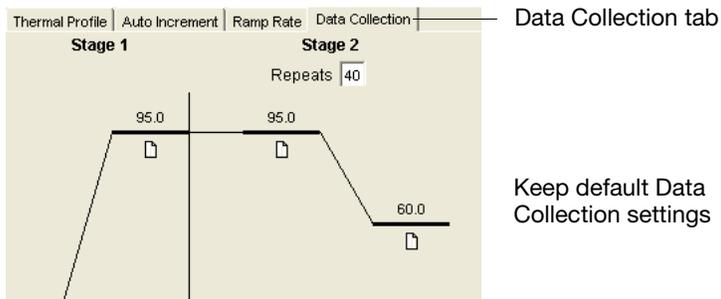


Note: If you choose to use AmpErase[®] UNG, you need to keep UNG activation step at the beginning of the thermal profile: 50 °C for 2 minutes.

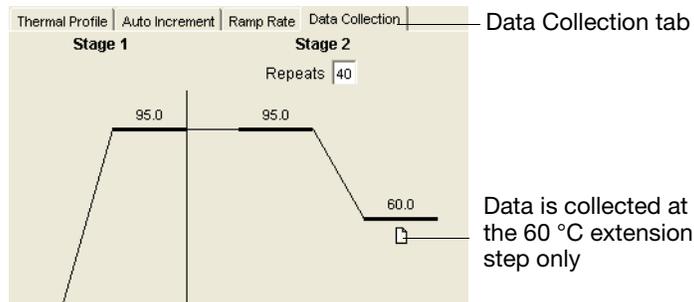
- Do not change the settings in the Auto Increment and Ramp Rate tabs. Keep the default settings as shown below.



- Select the Data Collection tab, then perform *one* of the following:
 - Change nothing in the Data Collection tab. The document icons indicate that data is collected in the associated stages.



- Alternatively, collect data only at the extension step by deselecting (clicking) the document icons in other stages.



6. Complete the plate document setup as described in:

- *Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* (PN 4364014)
or
- *Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide* (PN 4364016)
- *SDS Online Help*:

IMPORTANT! Set up the plate document to correspond exactly to the layout of samples in the wells of the reaction plate.

7. Save the plate document:

- a. Click  (or select **File > Save As**).
- b. For Files of Type, select **SDS 7900HT Document (*.sds)**.
- c. Navigate to where you want to save the plate document file.
- d. In the File Name field, enter a name for the plate document.
- e. Click **Save**.

Preparing Plates

For safety and biohazard guidelines, refer to the “Safety” section in the *TaqMan[®] Fast Universal PCR Master Mix (2X) Protocol* (PN 4351891). For all chemicals in **bold** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

For details on setting up the reactions, see the *TaqMan[®] Fast Universal PCR Master Mix (2X) Protocol* (PN 4351891).

IMPORTANT! The TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG, has a hot-start capability. However, to ensure optimal results, Applied Biosystems recommends running the reaction plate as soon as possible after completing the reaction setup. If you cannot run a reaction plate within 2 hours after completing the reaction setup, refrigerate or freeze the reaction plate until you can run it on the 7900HT Fast instrument.

1. Prepare the reaction mix for:

- a. **TaqMan[®] Gene Expression Assays**, according to the following table.

Component	Volume (μL) per One 20-μL Reaction	Volume (μL) per Four 20-μL Reactions [‡]
TaqMan [®] Gene Expression Assay (20X)	1.0	5.0
cDNA template (10 to 100 ng of cDNA) + RNase-free water	9.0	45.0 [§]
TaqMan [®] Fast Universal PCR Master Mix (2X), No AmpErase [®] UNG	10.0	50.0
Total Volume	20.0	100.0

[‡] Volumes are calculated for five reactions (rather than four) to compensate for the volume loss that occurs during reagent transfers.

[§] If you choose to use UNG, decrease the volume of cDNA template and RNase-free water to 8.8 μL per 20-μL reaction and add 0.2 μL of UNG stock (1 U/μL).

b. **Other quantitation assays**, according to the following table.

Component	Volume (µL) per One 20-µL Reaction	Volume (µL) per Four 20-µL Reactions‡	Final Concentration
TaqMan® Fast Universal PCR Master Mix (2X), No AmpErase® UNG	10.0	50.0	1X
Forward primer	2.0	10.0	50 to 900 nM
Reverse primer	2.0	10.0	50 to 900 nM
TaqMan® probe	2.0	10.0	50 to 250 nM
cDNA template§	2.0	10.0	10 to 100 ng
Nuclease-free water	2.0	10.0	-
Total Volume	20.0	100.0	-

‡ Volumes are calculated for five reactions (rather than four) to compensate for the volume loss that occurs during reagent transfers.

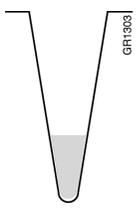
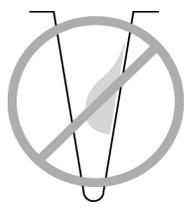
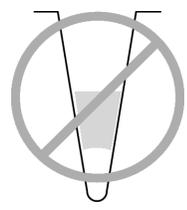
§ If you choose to use UNG, decrease the volume of cDNA template and RNase-free water to 8.8 µL per 20-µL reaction and add 0.2 µL of UNG stock (1 U/µL).

CAUTION CHEMICAL HAZARD. TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

2. Cap the tube(s), mix by gentle inversion, then centrifuge briefly.
3. Transfer 20 µL of reaction mix to wells of an optical 384-well plate.

IMPORTANT! The layout of the reactions (samples and assays) on the plate must match the arrangement (sample names and detectors/markers) in the plate document used for the run.

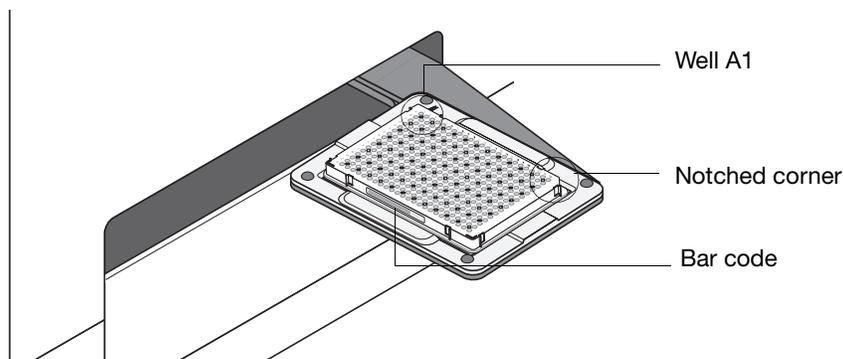
4. Seal the reaction plate with an optical adhesive cover:
 - MicroAmp® Optical Adhesive Film, quantity 100 (PN 4311971) *or*
 - MicroAmp® Optical Adhesive Film, quantity 25 (PN 4360954)
5. Centrifuge the plate briefly.
6. Verify that the reaction mix is at the bottom of the well.

Correct Position	Incorrect Positions	
 <p>The reaction mixture is at the bottom of the well.</p>	 <p>The reaction mixture is on the side wall because the plate was not centrifuged.</p>	 <p>An air bubble is at the bottom of the well because the plate was not centrifuged sufficiently.</p>

Running Plates **Running One Plate**

IMPORTANT! The TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG, provides a hot-start capability. However, to ensure optimal results, Applied Biosystems recommends running the reaction plate as soon as possible after completing the reaction setup. If you cannot run a reaction plate within 2 hours after completing the reaction setup, refrigerate or freeze the reaction plate until you can run it on the 7900HT Fast System.

1. Open the plate document in the SDS software.
2. Select the **Instrument** tab of the plate document, then select the **Real-Time** tab.
3. Verify that  is displayed in the status bar. If the software is not connected to the instrument, click **Connect to Instrument**.
4. (Optional). If the instrument tray is inside the instrument, click **Open/Close** to rotate the instrument tray to the OUT position.
5. Verify that the 384-well plate adapter is installed in the instrument tray.
6. Place the prepared reaction plate in the instrument tray with:
 - Well A1 at the top left corner of the tray and the notched corner at the top right.
 - The bar code toward the front of the instrument.



7. Click **Start Run**. The instrument tray rotates to the IN position. During the run, the instrument displays real-time status information in the **Instrument > Real-Time** tab and records the fluorescence emissions. Note that you can view the data generated in real-time during the amplification run.
8. When the run is complete and the Run Complete dialog box appears, click **OK** to close the dialog box, click **Open/Close**, then remove the plate from the instrument tray.

Running Multiple Plates

IMPORTANT! Applied Biosystems recommends running 384-well plates with TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG as soon as possible after completing the reaction setup. Therefore, when running multiple 384-well plates on the 7900HT Fast System, Applied Biosystems recommends that you load and unload the plates manually (without using the Automation Accessory).

Analyzing Results For information on analyzing the results, see the *Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* (PN 4364014) or *Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide* (PN 4364016).

Multiplex Applications **IMPORTANT!** Due to the complex nature of multiplex applications, assay performance cannot be guaranteed. However, the recommendations listed below should be helpful when running multiplex applications using Fast thermal cycling conditions and the TaqMan[®] Fast Universal PCR Master Mix (2X), No AmpErase UNG.

Perform the recommendations in the order listed:

1. Increase the annealing/extension temperature to 62 °C.
2. If you do not obtain the expected performance by increasing the annealing/extension temperature to 62 °C, then also increase the annealing/extension time in the thermal cycling protocol to 30 seconds.
3. If you do not obtain acceptable performance by increasing both the annealing/extension temperature and time, assay reoptimization may be required. Refer to the *Real-Time PCR Systems Chemistry Guide* (PN 4348358) for more information.

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