Performing Fast Gene Quantitation

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.

System Overview

Fast gene expression quantitation involves running TaqMan® Gene Expression Assays on the Applied Biosystems 7500 Fast Real-Time PCR System in less than 40 minutes.

System Requirements

- 7500 System with Fast hardware and software:
  - Fast 96-Well Block (thermal cycler)
  - SDS 1.4 Software for the 7500 Fast System
- Fast reagents and plastics:
  - 7500 Fast Spectral Calibration Kit I (PN 4360788)
  - 7500 Fast Spectral Calibration Kit II (PN 4362201)
  - Optical 96-Well Fast Thermal Cycling Plate with Barcode (code 128) (PN 4346906)
  - TaqMan® RNase P Fast 96-Well Instrument Verification Plate (PN 4351979)
  - TaqMan® Fast Universal PCR Master Mix (2X), No AmpErase® UNG (PN 4352042)

Getting Started

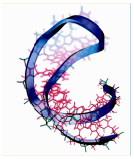
Before you perform Fast quantitation, make sure that you have:

- Installed the SDS 1.4 Software for the 7500 Fast System.
- Performed an ROI calibration.
- Performed a background calibration.
- Performed an optical calibration.
- Performed pure dye calibrations.
- Verified instrument performance.

Note: For calibration procedures refer to the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide* (PN 4347828).

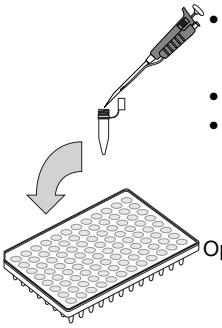
Workflow Summary

Create and Set Up a New Plate Document



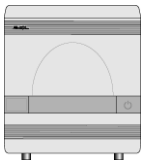
SDS 1.4 Software for the 7500 Fast System

Prepare Plate



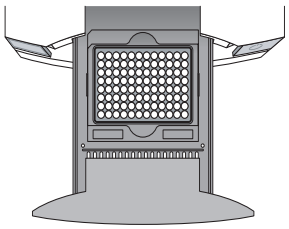
- TaqMan Fast Universal PCR Master Mix (25), No AmpErase UNG
- Primers and probes
- Template

Optical 96-Well Fast Plate



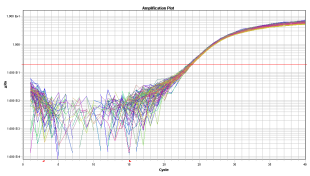
7500 Fast Real-Time PCR System

Run Plate



96-well plate in instrument drawer

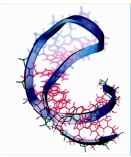
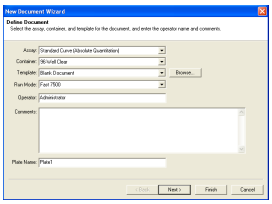
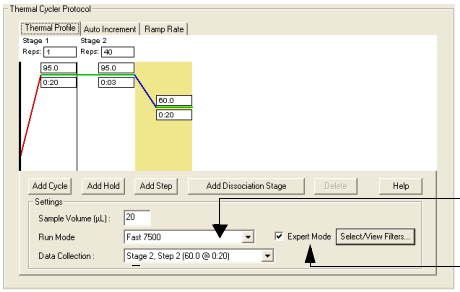
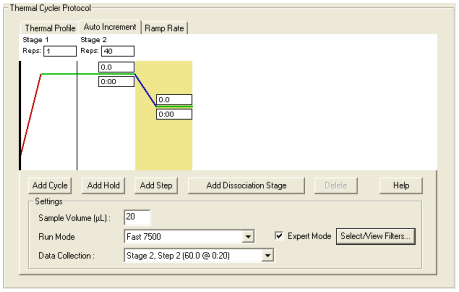
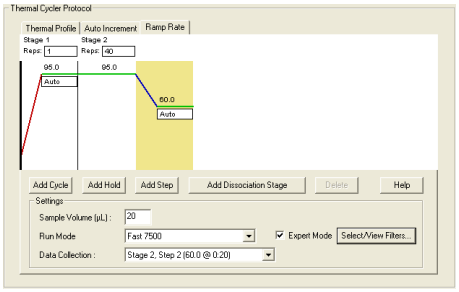
Analyze Results

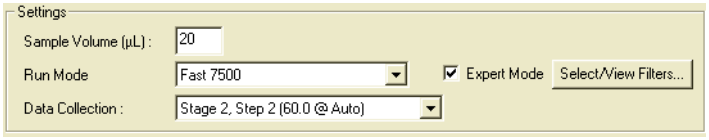
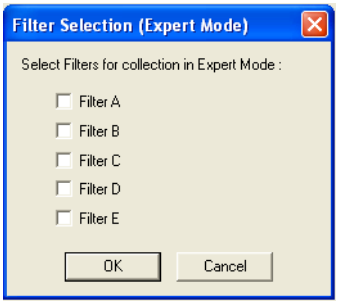
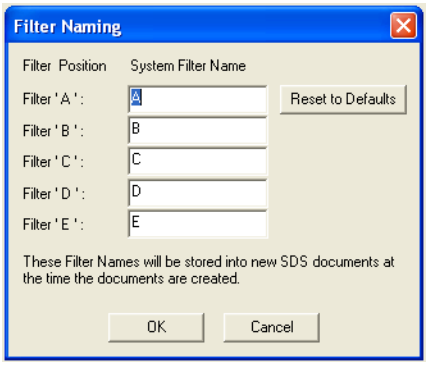


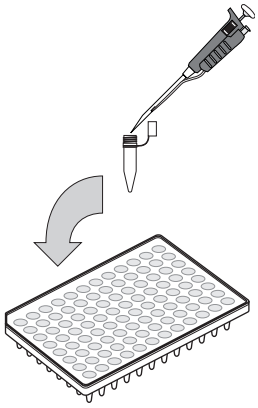
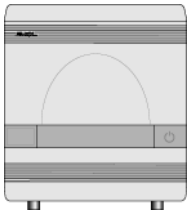
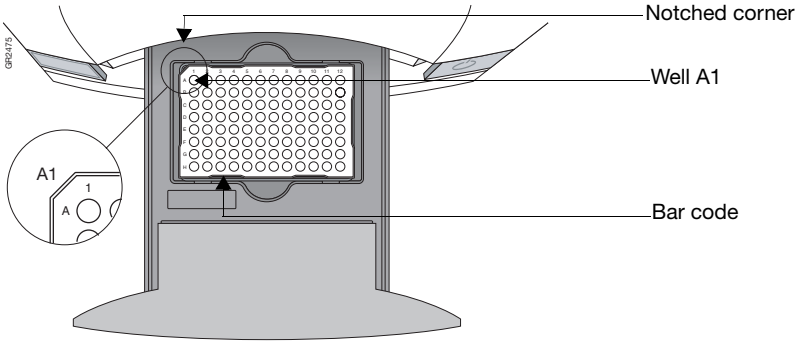
Amplification plot

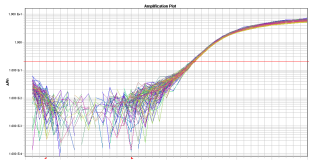
Procedures

For detailed procedures, see the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Getting Started Guide* (PN 4347825) and the *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantitation Getting Started Guide* (PN 4347824).

Step	Action	Description
1	Create and set up a new plate document.  SDS 1.4 Software for the 7500 Fast System	1. Select **Start > All Programs > Applied Biosystems > 7300/7500/7500 Fast System > 7300/7500/7500 Fast System Software.** 2. In the Quick Startup document dialog box, select **Create New Document**.  3. Complete the New Document Wizard page, then click **Next**.  4. Complete the plate setup: a. Create detectors and add to the plate document. b. Apply detectors and tasks for samples to the plate document. c. Add sample names to the plate document. 5. Select the **Instrument** tab, then verify that the default thermal cycler profile settings have the correct sample volume and conditions.  Default thermal cycler profile settings Fast mode selected Expert mode option **Note:** If you choose to use AmpErase® UNG, add an UNG activation step to the beginning of the thermal profile: 50 °C for 2 minutes.  Default auto increment settings  Default ramp rate settings

Step	Action	Description
	Create and set up a new plate document. *(continued)*	5. (optional) In Fast thermal cycling mode, select **Expert** mode to perform quantitation under 30 minutes using default thermal cycler profile settings. **Note:** Selection of the Fast thermal cycling mode results in default run times of under 40 minutes with data collection in all five filters. To run Expert mode: a. Verify the Run Mode is set to **Fast 7500**. b. Check the box marked **Expert Mode**.  c. Click **Select/View Filters**. The Filter Selection dialog box opens.  d. Check the filters you want to run in Expert mode. To rename the filters, select **OK** then select **Tools > Filter Configuration** and enter the desired name for each filter in the System Filter Name box.  **Note:** Applied Biosystems recommends the use of ROX™ dye to normalize any variation caused by pipetting error. **IMPORTANT!** Both FAM™ and ROX dye filters must be turned on in order to collect all data in the ROX passive reference dye. Use of more than three filters will require adjustment to the default extension time in order to complete data collection, and will increase the overall run time. e. Click **OK** and save the plate document as described below. 6. Save the plate document: a. Click (Save Document) or select **File > Save As**. b. Save as type **SDS Documents (*.sds)**. c. Navigate to the directory in which you want to save the plate document file. d. In the File name field, enter a name for the plate document. e. Click **Save**.

<table>
<tr><th>Step</th><th>Action</th><th>Description</th></tr>
<tr><td>2</td><td>Prepare plates.
  </td><td>
1. Prepare the reaction mix for each sample (for four 20-µL reactions):
<table>
<tr><th>Component</th><th>Volume (µL) /20-µL Reaction</th><th>Volume (µL) /Four 20-µL Reactions[a]</th></tr>
<tr><td>TaqMan® Gene Expression Assay (20×)</td><td>1.0</td><td>5.0</td></tr>
<tr><td>cDNA template (10 to 100 ng of cDNA) + RNase-free water</td><td>9.0[b]</td><td>45.0</td></tr>
<tr><td>TaqMan® Fast Universal PCR Master Mix (2×), No AmpErase® UNG</td><td>10.0</td><td>50.0</td></tr>
<tr><td>Total Volume</td><td>20.0</td><td>100.0</td></tr>
</table>
a Volumes are calculated for five reactions to provide excess volume for the loss that occurs during reagent transfers.
b If you choose to use UNG, decrease the volume of the 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).
2. Mix by gently pipetting up and down, cap the tube(s), and then centrifuge briefly.
3. Transfer 20 µL of reaction mix to each well of an Optical 96-Well Fast Thermal Cycling Plate.
4. Seal the plate with an optical adhesive cover, then centrifuge the plate briefly.
IMPORTANT! The TaqMan Fast Universal PCR Master Mix (2×) 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 load and run it on the 7500 Fast instrument.
</td></tr>
<tr><td>3</td><td>Run plate.
  </td><td>
1. If not already opened, open the plate document in the 7500 Fast System software.
2. If the instrument tray is inside the instrument, press firmly on the tray door to open.
3. Place the prepared reaction plate in the instrument tray so that Well A1 and the notched corner are in the top-left corner and the bar code is toward the front of the instrument.


4. Press firmly on the tray door to close.
5. Select the Instrument tab, then click Start.
</td></tr>
<tr><td>4</td><td>Analyze results.
  </td><td>
1. View the amplification plots.
2. Set the baseline and threshold values.
3. Use the standard curve method for absolute quantitation or the $\Delta\Delta C_T$ method for relative quantitation to analyze your data.
</td></tr>
</table>

Fast Gene Quantitation Products

Product	Applied Biosystems Part Number
Applied Biosystems 7500 Fast Real-Time PCR System	Contact your local Applied Biosystems sales office.
7500 System Fast Service Upgrade	Contact your local Applied Biosystems sales office.
7500 Fast Spectral Kit I	4360788
7500 Fast Spectral Kit II	4362201
Optical 96-Well Fast Thermal Cycling Plate with Barcode (code 128), 20 plates	4346906
Optical Adhesive Covers	4311971
SDS 1.4 Software for the 7500 Fast System	4363619
TaqMan® Fast Reagents Starter Kit	4352407
TaqMan® RNase P Fast 96-Well Instrument Verification Plate	4351979
TaqMan® Fast Universal PCR Master Mix (2X), No AmpErase® UNG	4352042
TaqMan® Gene Expression Assays	4331182

Related Documents

Document	Applied Biosystems Part Number
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Getting Started Guide	4347825
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantitation Getting Started Guide	4347824
Applied Biosystems Real-Time PCR Systems Chemistry Guide	4348358
TaqMan® Fast Universal PCR Master Mix (2X) Protocol	4351891
TaqMan® Gene Expression Assays Protocol	4333458

07/2006