

Performing Fast Gene Quantification

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 quantification involves running TaqMan® Gene Expression Assays on the Applied Biosystems 7900HT Fast Real-Time PCR System with Fast 96-Well Block Module in about 35 minutes.

System Requirements

- 7900HT System with Fast hardware and software:
 - Fast 96-Well Block (thermal cycler)
 - Fast 96-Well Plate Adapter
 - Upgraded 48V-650W power supply
 - Sequence Detection Systems Software v2.2.1
- Fast reagents and plastics:
 - 7900HT System Fast 96-Well Spectral Calibration Kit (PN 4351653)
 - 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 gene quantification, make sure that:

- The SDS software v2.2.1 is installed.
- The Fast 96-Well Block and the Fast 96-Well Plate Adapter are installed.
- A background run has been performed in the last 6 months.
- A pure dye run has been performed in the last 6 months.
- Instrument performance has been verified.

Note: For procedures, see the following documents:

- *Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification* (PN 4352533)
- *Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide* (PN 4351684).

Procedural Workflow

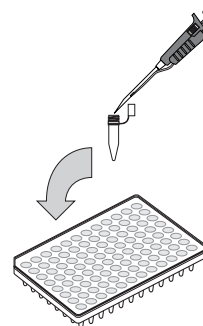
Creating and Setting
Up a New Plate
Document



SDS software v2.2.1



Preparing a Plate



- TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG
- TaqMan Gene Expression Assay
- cDNA template

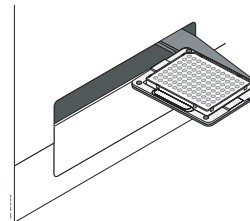
Optical 96-Well Fast Plate



Running a Single
Plate



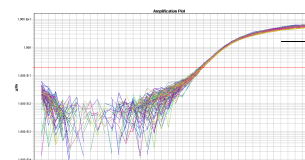
7900HT instrument
with Fast 96-Well Block
Module



Fast 96-Well
Plate Adapter



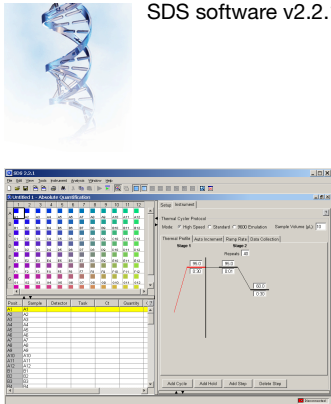
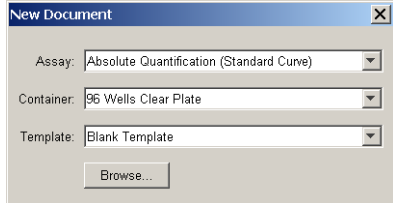
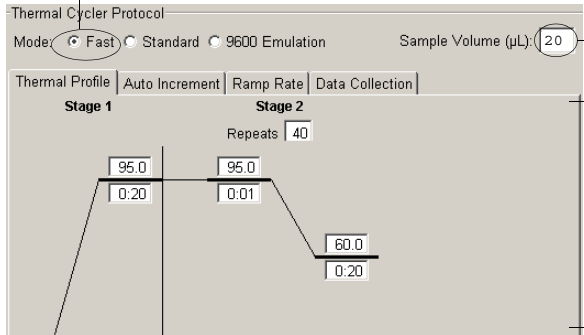
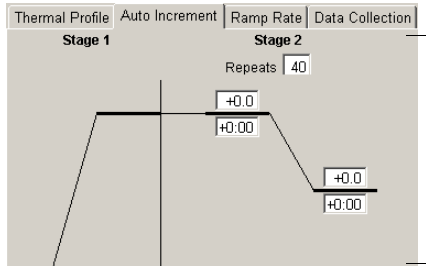
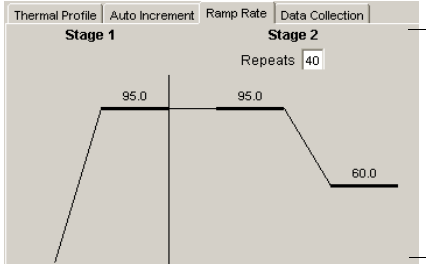
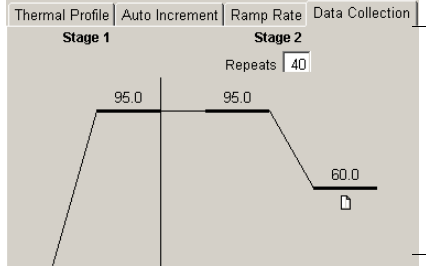
Analyzing Results


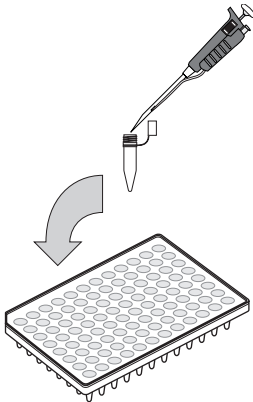


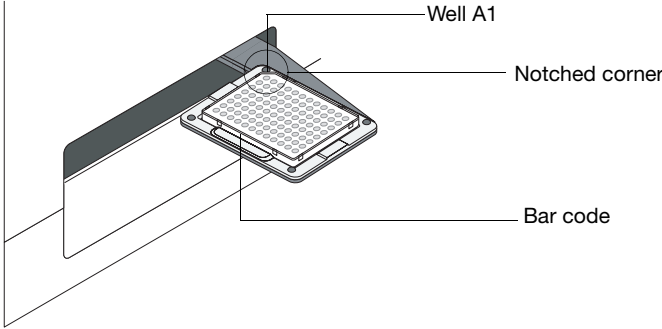
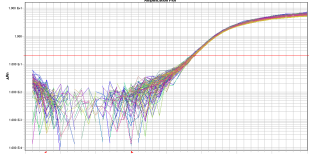


Amplification
plot

Procedures

For detailed procedures, see the *Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification* (PN 4352533) and the *Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide* (PN 4351684).

<div data-bbox="138 315 170 357">1</div>	<div data-bbox="224 310 511 359">Create and set up a new plate document.</div> <div data-bbox="370 373 560 399">SDS software v2.2.1</div> <div data-bbox="224 373 553 774"></div>	<div data-bbox="605 310 1380 373">1. Select Start > All Programs > Applied Biosystems > SDS 2.2.1 > SDS 2.2.1. 2. Select File > New, complete the New Document dialog box, then click OK:</div> <div data-bbox="605 384 995 585"></div> <div data-bbox="605 594 1414 651">3. Select the Instrument tab, then verify the default thermal cycler protocol settings: Fast mode is selected</div> <div data-bbox="605 659 1498 991"><div data-bbox="1209 684 1445 709">Sample Volume is 20 µL</div><div data-bbox="1209 873 1498 898">Default thermal profile settings</div></div> <div data-bbox="638 999 1507 1050"><p>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.</p></div> <div data-bbox="605 1066 1336 1329"><div data-bbox="1036 1186 1336 1211">Default auto increment settings</div></div> <div data-bbox="605 1350 1404 1612"><div data-bbox="1039 1465 1404 1512">Default ramp rate settings (cannot be modified with the Fast mode selected)</div></div> <div data-bbox="605 1633 1339 1896"><div data-bbox="1039 1745 1339 1770">Default data collection settings</div></div>
--	--	--

	Create and set up a new plate document (<i>continued</i>).	<ol style="list-style-type: none"> Complete setup: <ol style="list-style-type: none"> Create detectors (the first time only). Copy detectors to the plate document. Apply detectors and tasks for samples. Add sample names. Save the plate document: <ol style="list-style-type: none"> Click  (or select File > Save As). For Files of Type, select SDS 7900HT Document (*.sds). Navigate to where you want to save the plate document file. In the File Name field, enter a name for the plate document. Click Save. 															
2	Prepare plates. 	<ol style="list-style-type: none"> Prepare the reaction mix for each sample (for four 20-μL reactions): <table border="1" data-bbox="609 552 1507 814"> <thead> <tr> <th>Component</th><th>Volume (μL) /20-μL Reaction</th><th>Volume (μL) /Four 20-μL Reactions†</th></tr> </thead> <tbody> <tr> <td>TaqMan® Gene Expression Assay (20X)</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‡</td><td>45.0</td></tr> <tr> <td>TaqMan® Fast Universal PCR Master Mix (2X), 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> </tbody> </table> <p>† Volumes are calculated for five reactions to provide excess volume for the 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).</p> Cap the tube(s), mix by gentle inversion, then centrifuge briefly. Transfer 20 μL of reaction mix to wells of an Optical 96-Well Fast Plate. Seal the plate with an optical adhesive cover, then centrifuge the plate briefly. <p>IMPORTANT! The TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG, does provide 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 7900HT instrument.</p>	Component	Volume (μ L) /20- μ L Reaction	Volume (μ L) /Four 20- μ L 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
Component	Volume (μ L) /20- μ L Reaction	Volume (μ L) /Four 20- μ L 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															
3	Run a single plate. 	<ol style="list-style-type: none"> If not already opened, open the plate document in the SDS software. Select the Instrument tab, then select the Real-Time tab. Check if  Connected to 'PlateName' (Connected) is displayed in the status bar. If the software is not connected to the instrument, click Connect. If the instrument tray is inside the instrument, click Open/Close. Verify that the Fast 96-Well Plate Adapter is installed in the instrument tray. Place the prepared reaction plate into the instrument tray so that Well A1 and the notched corner are in the rear-left corner and the bar code is toward the front of the instrument.  <p>GR2383</p>															
4	Analyze results. 	<ol style="list-style-type: none"> View the amplification plots. Set the baseline and threshold values. Use the standard curve method or the relative quantification ($\Delta\Delta C_T$) method to analyze your data. 															



iScience. To better understand the complex interaction of biological systems, life scientists are developing revolutionary approaches to discovery that unite technology, informatics, and traditional laboratory research. In partnership with our customers, Applied Biosystems provides the innovative products, services, and knowledge resources that make this new, **Integrated Science** possible.

Fast Gene Quantification Products

Product	Applied Biosystems Part Number
Applied Biosystems 7900HT Fast Real-Time PCR System with Fast 96-Well Block Module	Contact your local Applied Biosystems sales office.
7900HT System Fast Service Upgrade	4351412
7900HT System Fast 96-Well Spectral Calibration Kit	4351653
Optical 96-Well Fast Thermal Cycling Plate with Barcode (code 128), 20 plates	4346906
Sequence Detection Systems Software v2.2.1	4352620
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
<i>Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide</i>	4351684
<i>Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification</i>	4352533
<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>	4333458

© Copyright 2004, Applied Biosystems. All rights reserved.

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER:

PLEASE REFER TO THE USER'S GUIDE FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

Practice of the patented polymerase chain reaction (PCR) process requires a license. The Applied Biosystems 7900HT Fast Real-Time PCR System base unit in combination with its immediately attached Fast 96-Well Block Module is an Authorized Thermal Cycler for PCR and may be used with PCR licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents.

Purchase of this instrument does not convey any right to practice the 5' nuclease assay or any of the other real-time methods covered by patents owned by Roche or Applied Biosystems.

Applied Biosystems, ABI PRISM and its design, and VIC are registered trademarks and AB (Design), Applera, JOE, NED, ROX, TAMRA, and TET are trademarks of Applera Corporation or its subsidiaries in the US and certain other countries.

AmpErase and TaqMan are registered trademarks of Roche Molecular Systems, Inc.

All other trademarks are the property of their respective owners.

09/2004