

Performing Fast Gene Quantitation

For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan*[®] *Fast Universal PCR Master Mix* (2×) *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 (2×), 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



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. | Select Start > All Programs > Applied Biosystems > 7300/7500/7500 Fast System > 7300/7500/7500 Fast System Software. | | |
| | SDS 1.4 Software for the 7500 Fast System | 2. In the Quick Startup document dialog box, select Create New Document. Image: Comparison of Comparison | | |
| | | 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. | | |
| | | Thema Profile Auto Incoments Flags Bugo 1 Bugo 2 Pref Flags Default | | |
| | | Setting Service Value (jul): Par Node Far 7000 Data Calection: Supp 2 Step 2 (60.0 @ 0.20) Cale Cale Cale Cale Cale Cale Cale Cale | | |
| | | 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 S | | |
| | | Image: Data Collection: | | |

| Step | Action | Description | | | |
|------|--|---|--|--|--|
| | Create and set up a new plate document. <i>(continued)</i> | (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 . | | | |
| | | | | | |
| | | Sample Volume (µL): 20 | | | |
| | | Data Collection : Stage 2. Step 2 (60.0 @ Auto) | | | |
| | | | | | |
| | | c. Click Select/View Filters. The Filter Selection dialog box opens. | | | |
| | | Filter Selection (Expert Mode) | | | |
| | | Select Filters for collection in Expert Mode : | | | |
| | | | | | |
| | | Filter C | | | |
| | | Filter D | | | |
| | | Filter E | | | |
| | | OK Cancel | | | |
| | | 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. | | | |
| | | Filter Namine | | | |
| | | | | | |
| | | Filter 'A ': | | | |
| | | Filter 'B ': B | | | |
| | | Filter "C": | | | |
| | | Filter ' D ' E | | | |
| | | These Filter Names will be stored into new SDS documents at | | | |
| | | the time the documents are created. | | | |
| | | OK Cancel | | | |
| | | 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 pageive reference due Use of more than these filters will acquire 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 . | | | |

| Step | Action | Description | | | |
|------|---|--|--|---|--|
| 2 | Prepare plates. | 1. Prepare the reaction mix for each sample (for four 20-µL reactions): | | | |
| | | Component | Volume (μL) /20-μL Reaction | Volume (μL) /Four 20-μL Reactions ^a | |
| | | TagMan [®] Gene Expression Assay (20X) | 1.0 | 5.0 | |
| | | cDNA template (10 to 100 ng of cDNA) + RNase-free water | 9.0 ^b | 45.0 | |
| | | TaqMan [®] Fast Universal PCR Master Mix (2×), No AmpErase [®] UNG | 10.0 | 50.0 | |
| | | Total Volume | 20.0 | 100.0 | |
| | 2 A A A A A A A A A A A A A A A A A A A | a Volumes are calculated for five reactions to provide excess volu reagent transfers. b If you choose to use UNG, decrease the volume of the cDNA tem per 20-μL reaction and add 0.2 μL of UNG stock (1 U/μL). | me for the loss tha plate and RNase-f | t occurs during ree water to 8.8 μL | |
| | | 2. Mix by gently pipetting up and down, cap the tube(s), and t | hen centrifuge b | riefly. | |
| | | 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 M provides a hot-start capability. However, to ensure optimal recommends running the reaction plate as soon as possible setup. If you cannot run a reaction plate within 2 hours after refrigerate or freeze the reaction plate until you can load an instrument. | ix (2X) No AmpE results, Applied e after completin r completing the d run it on the 75 | Trase UNG Biosystems Ig the reaction reaction setup, 500 Fast | |
| 3 | Run plate. | 1. If not already opened, open the plate document in the 7500 Fast System | | oftware. | |
| | - Maga. | 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. | | | |
| | | | Well A1 | d corner | |
| | | | Bar co | de | |
| | | 4. Press firmly on the tray door to close. | | | |
| | | 5. Select the Instrument tab, then click Start . | | | |
| 4 | | View the amplification plots. Set the baseline and threshold values | | | |
| | | Set the baseline and threshold values. Use the standard curve method for absolute quantitation or the ΔΔC_T method for rel quantitation to analyze your data. | | | |

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 (2×) Protocol | 4351891 |
| TaqMan [®] Gene Expression Assays Protocol | 4333458 |

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PLEASE REFER TO THE APPLIED BIOSYSTEMS 7300/7500/7500 FAST GETTING STARTED GUIDES FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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