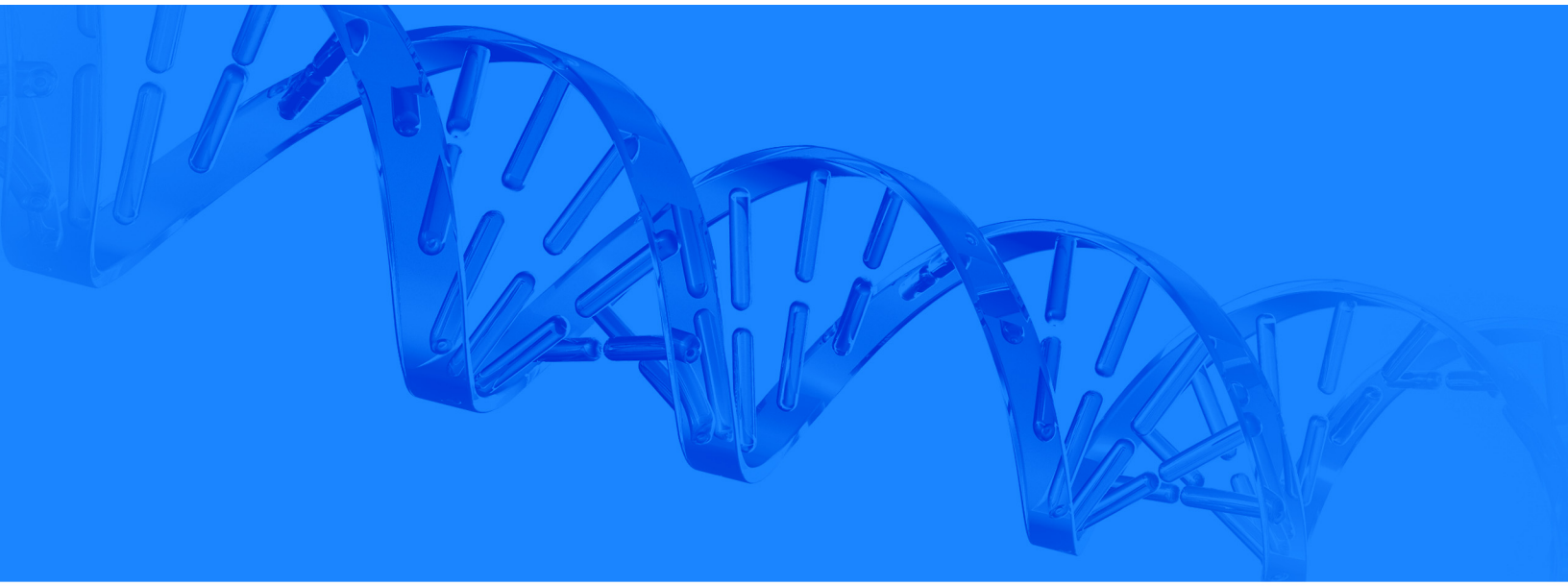
Applied Biosystems

TaqMan® Gene Expression Assays



Applied Biosystems

TaqMan® Gene Expression Assays

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Contents

How to use this guide

Purpose of this guide

The *TaqMan® Gene Expression Assays Protocol* provides step-by-step instructions for performing and analyzing experiments using TaqMan® Gene Expression Assays.

Audience

This protocol is intended for novice and experienced laboratory personnel who perform experiments using TaqMan® Gene Expression Assays.

Assumptions

This guide assumes that you have a working knowledge of:

- General techniques for handling RNA samples and preparing them for RT-PCR.
- The thermal cycler and the real-time PCR system that you use to perform the RT-PCR.

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Enter **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Safety information

Note: For general safety information, see this Preface and Appendix D, "Safety," on page 43. When a hazard symbol and hazard type appear in this document with a chemical name or instrument hazard, see "Chemical alerts" on page 47 for the complete safety text on the chemical or instrument hazard.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

WARNING! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "Obtaining MSDSs" on page 45.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

TaqMan® Gene Expression Assays

Product information

Purpose of the product

TaqMan® Gene Expression Assays are a comprehensive collection of predesigned, preformulated gene-specific primer and probe sets that help researchers perform quantitative gene expression studies on human, bovine, canine, mouse, rat, rhesus, zebrafish, *Arabidopsis*, *C. elegens*, and *Drosophila* genes.

The following TaqMan® Gene Expression Assays are available:

- **Inventoried Assays** – Predesigned real-time PCR assays that are previously manufactured and immediately available at the time you submit an order.
- **Made-to-Order Assays** – Predesigned real-time PCR assays that are manufactured at the time you submit an order.
- **Custom Assays** – Custom assays designed for you to target any sequence within a gene, either across exon boundaries or within an exon. Submit a target sequence for any organism, and Applied Biosystems sends you a ready-to-use gene expression assay with optimized primers and probe.
- **TaqMan® Endogenous Controls** – A collection of predesigned assays for candidate control genes used to normalize for differences in sample RNA added to a reaction. A number of candidate endogenous control genes are available for use with TaqMan® Gene Expression Assays. For more information on selecting endogenous controls, see "Step 3: Order a candidate endogenous control assay" on page 25.

Note: For information about the mechanics of the TaqMan® assays, refer to "About TaqMan® chemistry" on page 36.

Compatible real-time instruments

TaqMan® Gene Expression Assays can be used with the Applied Biosystems:

- 7300 Real-Time PCR System
- 7500 Real-Time PCR System
- 7500 Fast Real-Time PCR System
- 7900HT Real-Time PCR System
- StepOne™ Real-Time PCR System
- StepOnePlus™ Real-Time PCR System

Kit contents

TaqMan® Gene Expression Assays include:

- One tube for each assay that is ordered. The tube contains:
  - Two unlabeled primers (1× final concentration is 900 nM per primer; 20× stock concentration is 18 µM per primer)
  - One 6-FAM™ dye-labeled, TaqMan® MGB probe (1× final concentration is 250 nM; 20× stock concentration is 5 µM)

Note: The assay ID that appears on the tube of each TaqMan® Gene Expression Assay is a unique, alphanumeric string that identifies the assay and encodes basic descriptive information. See "About TaqMan® Gene Expression Assay IDs" on page 21 for more information.

- A data sheet containing information about the assay.
- An Information CD that contains:
  - An assay information file (AIF)
  - A *TaqMan® Gene Expression Assays Protocol* (PN 4333458)
  - A *TaqMan® Gene Expression Assays Quick Reference Card* (PN 4401212)

Note: For more information on the Information CD, see "About the TaqMan® Gene Expression Assays Information CD" on page 38.

TaqMan® Gene Expression Assays	No. of 20-µL reactions	Part number	Availability
Inventoried (20×)‡	250	4331182	In stock
Made-to-Order (20×)	360	4351372	Manufactured when ordered
Custom (60×)	2900	4332079	
Custom (20×)	750	4332078	
	360	4331348	
TaqMan® Endogenous Controls (20×)	50 200 1000	See "Step 3: Order a candidate endogenous control assay" on page 25.	• Non-primer limited, FAM™ dye-labeled MGB probe • Primer limited, VIC® dye-labeled MGB probe • Primer limited, VIC® dye-labeled, TAMRA™ dye-quenched probe

‡ TaqMan® Gene Expression Assays are preformulated at 20×.

Order and store the TaqMan® Gene Expression Assays

Ordering an assay

For details on how to order an assay, refer to the TaqMan® Gene Expression Assays products page at **www.allgenes.com** or Appendix A, "How to Order TaqMan® Gene Expression Assays," on page 19.

Select an endogenous control

For a list of endogenous control assays, refer to "Step 3: Order a candidate endogenous control assay" on page 25. For more information on selecting an endogenous control, refer to the Application Note: *Using TaqMan® Endogenous Control Assays to Select an Endogenous Control for Experimental Studies* (Stock Number 127AP08-01) available at **www.appliedbiosystems.com**

Storage and dilution

- Store the TaqMan® Gene Expression Assays at –15 to –25 °C and keep them protected from light.
- To minimize freeze-thaw cycles, consider diluting 60× assays to a 20× working stock and aliquoting the solution into smaller volumes.

Materials and equipment not included

Obtain the following materials for the reverse transcription and PCR (see "Step 5: Order materials and equipment not included" on page 29 for a complete list of materials). Unless otherwise indicated, all materials are available from major laboratory suppliers (MLS).

Table 1 Required materials and equipment

✔	Material	Source
	Reverse transcription reagents	Applied Biosystems (see Table 10 on page 29)
	PCR reagents	
	Thermal cycler (or real-time PCR instrument)	Applied Biosystems
	Real-Time PCR Instrument	
	Reaction consumables	Applied Biosystems (see Table 11 on page 30)
	Centrifuge (with plate adapter)	MLS
	Disposable gloves	MLS
	Microcentrifuge	MLS
	Pipette tips, aerosol-resistant	MLS
	Pipettors (positive/air-displacement or multichannel)	MLS
	Polypropylene tubes (various sizes)	MLS
	Vortexer	MLS
	Nuclease-free water (no diethyl pyrocarbonate [DEPC])	MLS

Workflow

Figure 1 shows a simplified workflow for using TaqMan® Gene Expression Assays.

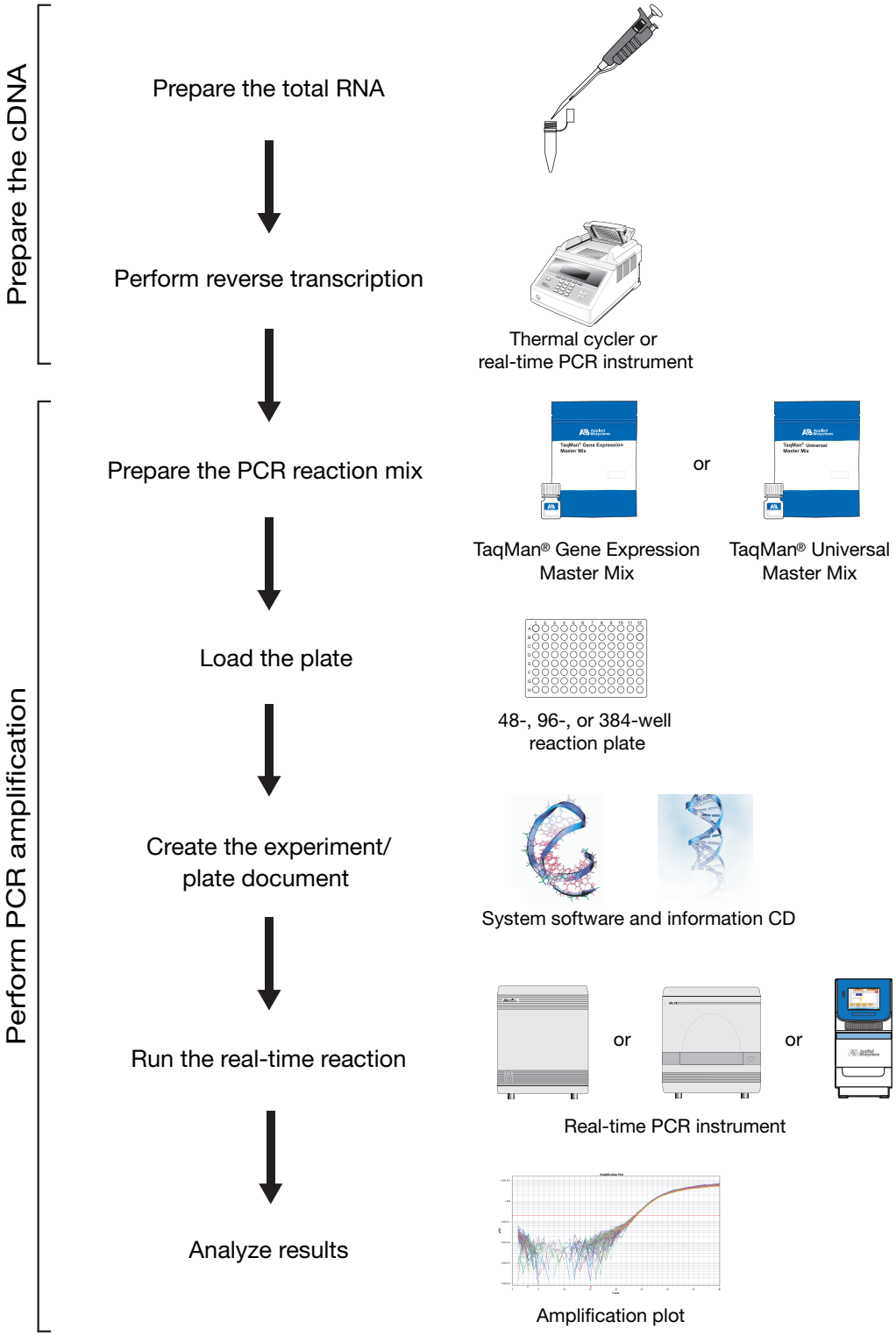


Figure 1 TaqMan® Gene Expression Assays workflow

Prepare the cDNA sample

Prepare the total RNA

Before running the TaqMan® Gene Expression Assays, synthesize single-stranded cDNA from total RNA samples. For optimal performance, Applied Biosystems recommends using a RNA isolation kit from Ambion® Inc. Go to **www.ambion.com** to view a list of kits.

Applied Biosystems recommends using RNA that is:

- Between 0.002 and 0.2 µg/µL
- Less than 0.005% of genomic DNA by weight
- Dissolved in a PCR-compatible buffer
- Free of RNase activity
- Free of inhibitors of reverse transcription and PCR
- Nondenatured

IMPORTANT! Denaturation of the RNA is not necessary and may reduce the yield of cDNA for some gene targets.

Perform reverse transcription

Applied Biosystems recommends the High-Capacity cDNA Reverse Transcription Kit to obtain cDNA from RNA samples. Refer to the *High-Capacity cDNA Reverse Transcription Protocol* (PN 4375575) for details.

Note: See "Related reagents" on page 29 for a list of kit part numbers.

Evaluate the cDNA

Applied Biosystems recommends that you use:

- 1 to 100 ng of cDNA per 20-µL reaction
- The same amount of cDNA for all samples

DNA quantitation methods

Applied Biosystems recommends two DNA quantitation methods:

- UV absorbance (A_{260}/A_{280}) measurements

  or
- The TaqMan® RNase P Detection Reagents (PN 4316831). You can use your own DNA samples or the TaqMan® DNA Template Reagents (PN 401970) to create a standard curve. Refer to *Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR* (PN 4371090). You can download the document from **docs.appliedbiosystems.com/search.taf**

Note: The TaqMan® RNase P method is preferred to the UV absorbance method because it is more accurate and it assesses sample quality.

(Optional) Store the cDNA

If you do not proceed immediately to PCR amplification, store all cDNA samples at –15 to –25 °C. To minimize freeze-thaw cycles, aliquot the samples.

Prepare the reaction mix and load the plate

Thaw and mix the reagents

1. Thaw on ice, resuspend completely by gently vortexing, then centrifuge briefly:
   - TaqMan® Gene Expression Assay (20×)
   - cDNA samples
2. Mix the master mix reagent by gently swirling the bottle. (See "Related reagents" on page 29 for a list of compatible master mixes available from Applied Biosystems.)

Calculate the number of reactions

Calculate the number of reactions that you need for each assay. Applied Biosystems recommends performing four replicates of each reaction. Be sure to include on each plate:

- A gene expression assay for each cDNA sample
- Endogenous control assays
- (Optional) No template controls (NTCs) for each gene expression assay on the plate

Prepare the PCR reaction mix

For the following hazard, see the complete safety alert description in Appendix D, "Safety" on page 43.

CAUTION! CHEMICAL HAZARD. TaqMan® Gene Expression Master Mix, TaqMan® Universal PCR Master Mix (2×; with or without AmpErase® UNG), TaqMan® Fast Universal PCR Master Mix (2×), No AmpErase® UNG

1. Obtain a sterile 1.5-mL microcentrifuge tube for each sample (to be run in quadruplicate).
2. Pipette the following into each tube:

PCR reaction mix component	Volume per 20-µL reaction (µL)	
	Single reaction	Four replicates‡
20× TaqMan® Gene Expression Assay	1.0	5.0
cDNA template§ + RNase-free water	9.0	45.0
2× TaqMan® Gene Expression Master Mix#	10.0	50.0
Total Volume	20.0	100.0

‡ Replicate volumes include 20% excess for volume loss from pipetting.
§ To each 20-µL reaction, add 1 to 100 ng cDNA template diluted to the correct volume using RNase-free water.
# (Optional) Use TaqMan® Fast Universal Master Mix (2×), No AmpErase® UNG or TaqMan® Universal Master Mix. If you add AmpErase® UNG (uracil-N-glycosylase), the final concentration must be 0.01 U/µL. To compensate for additional volume from the UNG, decrease proportionally the volume of water used to dilute the cDNA template. See "Related reagents" on page 29 for a complete list of master mixes available from Applied Biosystems.

3. Cap the tube and invert several times to mix.
4. Centrifuge the tube briefly.

Load the plate

1. Select a 48-, 96-, or 384-well plate and plate cover. Refer to "Reaction plates and accessories" on page 30 for a list of compatible consumables.
2. Transfer 20 µL of PCR reaction mix into each well of the reaction plate.
3. Seal the plate with the appropriate cover.
4. Centrifuge the plate briefly.

IMPORTANT! If you use TaqMan® Fast Universal PCR Master Mix (2×), run the reaction plate within 2 hours of completing the reaction setup. Otherwise, refrigerate or freeze the plate until you can load it into the instrument.

Note: If you perform the run on a 7900HT Fast system with a 96-well Fast block and an automation accessory, place a MicroAmp® Snap-On Optical Film Compression Pad (PN 4312639) on top of the plate.

5. Load the plate into the instrument.

Run the real-time PCR reaction

Create the plate document/ experiment and run the plate

1. Create an plate document/experiment for the run using the parameter values shown in Table 2.
2. Run the plate.

   For instructions on how to create and run an plate document/experiment, see "Related documentation" on page 51 for a list of documents for your instrument.

Table 2 Plate document/experiment parameters

System	Run	Reaction plate‡	Ramp speed or mode	Sample volume (µL)	Thermal cycling conditions
7300/7500	Standard	96-well standard	Standard§	20	Table 3 below
7500 Fast	Standard	96-well Fast	Standard	20	Table 4 on page 9
	Fast	96-well Fast	Fast		
7900HT Fast	Standard	96-well standard	Standard	20	Table 5 on page 9
		384-well standard			
	Fast	96-well Fast	Fast		
		384-well standard			
StepOne™	Standard	48-well Fast	Standard	20	Table 6 on page 9
	Fast	48-well Fast	Fast		
StepOnePlus™	Standard	96-well Fast	Standard	20	
	Fast	96-well Fast	Fast		

‡ See Table 11 on page 30 for a list of consumables for Applied Biosystems real-time PCR systems.
§ The 7300 system has only one run mode (Standard 7300).

IMPORTANT! If you use a third-party real-time PCR instrument to perform the PCR, use the standard thermal cycling protocol to run the plate (see Table 3). For more information, refer to the documentation for your instrument.

Thermal cycling conditions

Table 3 7300/7500 system thermal cycling conditions

Run type	Reaction plate	Stage	Temp (°C)	Time
Standard	96-well standard	Hold‡	50	2 min
		Hold	95	10 min
		Cycle (40 cycles)	95	15 sec
			60	1 min

‡ Required for optimal AmpErase® UNG activity; not needed when UNG is not in the reaction.

Table 4 7500 Fast system thermal cycling conditions

Run type	Reaction plate	Stage	Temp (°C)	Time
Standard	96-well Fast	Hold‡	50	2 min
		Hold	95	10 min
		Cycle	95	15 sec
		(40 cycles)	60	1 min
Fast	96-well Fast	Hold‡	50	2 min
		Hold	95	20 sec
		Cycle	95	3 sec
		(40 cycles)	60	30 sec

‡ Required for optimal AmpErase® UNG activity; not needed when UNG is not in the reaction.

Table 5 7900HT system thermal cycling conditions

Run type	Reaction plate	Stage	Temp (°C)	Time
Standard	96- or 384-well standard	Hold‡	50	2 min
		Hold	95	10 min
		Cycle	95	15 sec
		(40 cycles)	60	1 min
Fast	96-well Fast	Hold‡	50	2 min
		Hold	95	20 sec
	384-well standard	Cycle	95	1 sec
		(40 cycles)	60	20 sec

‡ Required for optimal AmpErase® UNG activity; not needed when UNG is not in the reaction.

Table 6 StepOne™/StepOnePlus™ system thermal cycling conditions

Run type	Reaction plate	Stage	Temp (°C)	Time
Standard	48- or 96-well Fast	Hold‡	50	2 min
		Hold	95	10 min
		Cycle	95	15 sec
		(40 cycles)	60	1 min
Fast	48- or 96-well Fast	Hold‡	50	2 min
		Hold	95	20 sec
		Cycle	95	1 sec
		(40 cycles)	60	20 sec

‡ Required for optimal AmpErase® UNG activity; not needed when UNG is not in the reaction.

Analyze the results

Analyzing the data from TaqMan® Gene Expression Assays requires you to:

- View the amplification plots for the entire plate
- Set the baseline and threshold values
- Use the relative standard curve or the comparative C_T method to analyze your data

The details of the data analysis depend on the real-time PCR instrument that you use. Refer to the appropriate instrument user documentation for instructions on how to analyze your data.

Resources for data analysis

For more information about analyzing your data, refer to the user guide for your real-time PCR instrument.

Real-time PCR system	Document	Part number
7900HT Fast system	*Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantification Getting Started Guide*	4364014
	Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantification Getting Started Guide	4364016
	Applied Biosystems 7900HT Fast Real-Time PCR System Quick Reference Card: Performing Fast Gene Quantification	4351892
	Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification	4352533
7300/7500/ 7500 Fast system	*Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantification Getting Started Guide*	4347825
	Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantification Getting Started Guide	4347824
	Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments	4387779
	Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Comparative C_T/Relative Standard Curve Experiments	4387783
StepOne™/ StepOnePlus™ system	*Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Getting Started Guide for Standard Curve Experiments*	4376784
	Applied Biosystems StepOne™/StepOnePlus™ Real-Time PCR System Getting Started Guide for Comparative C_T/Relative Standard Curve Experiments	4387783
All	*Real-Time PCR Systems Chemistry Guide: Applied Biosystems 7900HT Fast Real-Time PCR Systems and 7300/7500/7500 Fast Real-Time PCR Systems*	4348358

Troubleshooting

Table 7 Troubleshooting

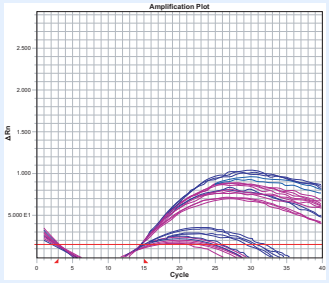
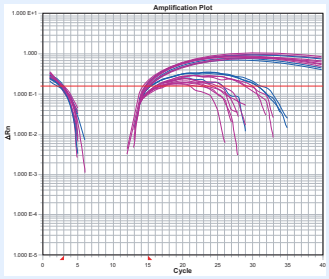
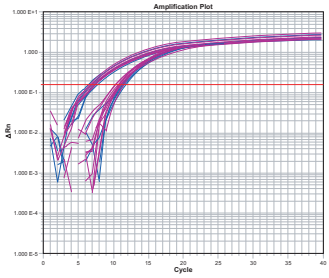
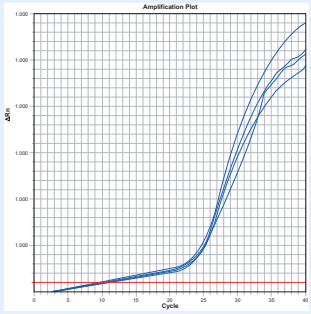
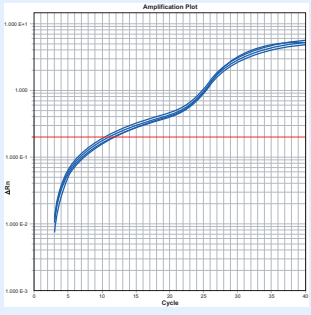
Observation	Possible cause	Recommended action
Amplification curve shows abnormal plot and/or low ΔR_n values. Linear view:  Log view: 	The baseline was improperly set (some samples have C_T values lower than the baseline stop value).	Refer to your real-time PCR system user guide for procedures on setting the baseline. Switch from manual to automatic baselining, or move the baseline stop value to a lower C_T (2 cycles before the amplification curve for the sample crosses the threshold). Log view corrected: 
	An amplification signal is detected in the early cycles (no baseline can be set because the signal is detected too early).	Dilute the sample to increase the C_T value.
Amplification curve shows a rising baseline. Linear view:  Log view: 	Primer and probe interaction	• Adjust the threshold manually. • Select another assay from the same gene.

Table 7 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
Amplification curve shows weak amplification.	Sequence mismatches between target and assay sequences	Perform bioinformatics. For more information, refer to the: • *Custom TaqMan® Genomics Assays Protocol: Submission Guidelines* (PN 4367671) • *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (from **www.appliedbiosystems.com**)
	Degraded reagents and/or probe	• Check the expiration date of the reagents. • Make sure you follow the correct handling and storage conditions. • Avoid excessive freeze-thaw cycles. (Consider diluting the 60× TaqMan® Gene Expression Assay to a 20× working stock.)
	Degraded or contaminated template	• Improve the sample integrity (extraction methods). See "Prepare the cDNA sample" on page 5. • Check each template preparation by agarose gel electrophoresis or bioanalyzer to determine the: – Purity (only one product should be formed) – Level of degradation • Use RNase-free, sterile, filtered water.
	Inhibitors present in the reaction	• Verify the presence of an inhibitor: a. Create a serial dilution of your sample. b. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, low concentrations yield higher-than-expected C_T values. (High concentration means more inhibition because the sample is not diluted.) c. Rerun the assay with purified template. • Improve sample integrity (extraction methods). See "Prepare the cDNA sample" on page 5.
	Poor reverse transcription (RT) conversion to cDNA	• Check the RNA sample for degradation. • Input RNA could be too concentrated or too dilute. Verify the concentration by optical density (OD), make new serial dilutions of template RNA from original stock, then repeat the RT-PCR. • Ensure that the RT-PCR setup is performed under the appropriate conditions to avoid premature cDNA synthesis. • Check the RT reagents for contamination and/or degradation.
	Primer-dimer formation and residual polymerase activity	(*Fast chemistry only*) For optimal results, run 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.
Amplification curve shows low ROX™ dye (passive reference dye).	Inaccurate pippetting: Little or no TaqMan® Universal PCR Master Mix	Follow accurate pipetting practices.

Table 7 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (C_T = 40) across all assays or in an unusually large number of assays.	One or more of the reaction components was not added.	Make sure the cDNA, TaqMan® Gene Expression Assay, and TaqMan® Gene Expression Master Mix were added to the reaction plate. (If the master mix is missing, the passive reference fails.)
	Incorrect dye components were selected.	Check the dye components settings and reanalyze the data.
	The annealing temperature on the thermal cycler was too high for the primers and/or probe.	Make sure the thermal cycler is set to the correct annealing and extension temperatures. Ensure that the thermal cycler is calibrated and maintained regularly.
	Inappropriate reaction conditions were used.	Troubleshoot the RT-PCR optimization.
	Degraded template	• Determine the quality of the template. • Rerun the assay with fresh template. • Use RNase-free reagents. • Use an RNase inhibitor.
	Inhibitors present in the reaction	Verify the presence of an inhibitor: 1. Create a serial dilution of your sample. 2. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, low concentrations yield higher-than-expected C_T values. (High concentration means more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template.
	The baseline and/or threshold was improperly set	Refer to your real-time PCR system user guide for procedures on setting the baseline and threshold: • Switch from automatic to manual baselining, or from manual to automatic. • Lower the threshold value to within the appropriate range.
	Assay design or synthesis failure: The wrong sequence was submitted to Applied Biosystems.	• Verify that the sequence that you submitted is correct. • Check for an alternative transcript or a splice variant.
	Assay is designed in a variable region of the gene transcript.	Verify that the location targeted by the assay is not within the 5′ untranslated region (UTR), which can be highly variable between transcripts. If the assay is designed within the 5′ UTR, select a different assay that is within the coding region of the transcript. Otherwise, select an assay for an alternative transcript or splice variant.
	cDNA conversion failed.	• Check the RNA integrity and concentration. • Check for RNase activity. • Follow Applied Biosystems recommended thermal profile. • Repeat the RT step with new reagents.

Table 7 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
Amplification curve shows samples within the same assay that have differently shaped curves.	The baseline was set improperly.	Refer to your real-time PCR system user guide for procedures on setting the baseline: • Switch from automatic to manual baselining, or from manual to automatic. • Increase the upper or lower value of the baseline range.
	Sample quality is poor.	1. Perform a quality check on the sample. 2. If necessary, reextract the sample.
	Imprecise pipetting: different concentrations	Follow accurate pippetting practices.
	Contamination	Be sure your workspace and equipment are properly cleaned.
Amplification curve shows no amplification of the sample (C_T = 40) in the target assay.	One or more of the reaction components was not added.	Check your pipetting equipment and/or technique.
	Incorrect dye components were selected.	Check the settings of the dye components before data analysis.
	The gene is not expressed in the tested sample.	• Verify by: – Rerunning the sample using the same assay – Running the sample using an alternative assay for the same gene • Verify the known expression of the gene in the sample type. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	The reaction may not have enough copies of the target gene.	Verify by: • Rerunning the sample using the same assay • Rerunning the assay using more sample • Running the sample using an alternative assay for the same gene **Note:** If the recommended actions do not resolve the problem, the result may be correct.
Decrease in ROX™ dye fluorescence (passive reference dye)	Precipitation in the TaqMan® buffers	• When using the TaqMan® PCR Core Reagents Kit, be sure to mix the tubes well. • Use TaqMan® Gene Expression Master Mix (2×). Be sure to mix thoroughly to produce a homogenous solution.
	Degraded TaqMan® buffers	Verify that the kits have been stored according to the instructions on the packaging and have not expired.
Simultaneous increase in fluorescence from both the: • Passive reference (ROX™) dye • Reporter dye(s)	Evaporation	Check the seal of the optical adhesive cover for leaks.
Multicomponent signal for ROX™ dye is not linear.	Pure dye components spectra are incorrect.	Rerun the pure dye spectra.
	Incorrect dye components were selected.	Select the correct dyes for the data analysis.

Table 7 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
R_n on R_n-vs.-Cycle plot is very high.	ROX™ dye was not selected as the passive reference when the plate document/ experiment was set up.	Select the ROX™ dye as the passive reference, then reanalyze the data.
No template control (NTC) shows amplification.	Contaminated reagents (contaminated with gDNA, amplicon, or plasmid clones)	• Rerun the assay using new reagents. • Be sure your workspace and equipment are cleaned properly. • Use AmpErase® UNG. • Run no-reverse-transcription controls to rule out genomic DNA contamination. • (*gDNA contamination only*) Design an assay that spans an exon-exon boundary.
	Bacterial sequences used as template.	Use AmpliTaq Gold® LD DNA Polymerase.
Standard curve: • Poor slope (a slope value of –3.32 equals approximately 100% efficiency) *or* • Poor correlation coefficient (the best correlation coefficient is 1.0)	Incorrect dilutions	• Redilute the samples. Ensure pipettes are calibrated. • Pipette more than 5 µL of sample.
	Inaccurate pipetting	• Check the calibration of the pipettes. • Pipette more than 5 µL of sample.
	Inhibitors present in the reaction	Verify the presence of an inhibitor: 1. Create a serial dilution of your sample. 2. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, low concentrations yield higher-than-expected C_T values. (High concentration means more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template.
	Improper reaction conditions	Follow the Applied Biosystems recommended thermal cycling profile.
	Inconsistent replicates (high standard deviation)	Make a master mix for each dilution point on the curve, then transfer to the reaction plate.
	Range of dilution points is too narrow.	Increase the number of points and the logarithmic range.
	Incorrect baseline and threshold settings	Verify settings according to your real-time PCR system user documentation.
	(*Bad correlation coefficient only*) Improper mixing	• Increase the length of time that you mix the reagents. • Make a master mix for each dilution point on the curve, then transfer to the reaction plate.
The endogenous control C_Ts vary, or do not normalize the sample well.	Endogenous control is not consistently expressed across the samples.	See "Select an endogenous control" on page 3 for information on selecting an endogenous control.
	Sample concentrations vary widely	If desired, quantitate and normalize samples before running them.
	Inaccurate pipetting	• Check the calibration of the pipettes. • Pipette more than 5 µL of sample.

Table 7 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
High standard deviation of replicates (inconsistent data, C_T varies).	Inefficient mixing of reagents	• Increase the length of time that you mix the reagents. • Make a master mix for each dilution point on the curve, then transfer to the reaction plate. • Validate your mixing process by running a replicate plate.
	Inaccurate pipetting	• Check the calibration of the pipettes. • Pipette more than 5 µL of sample.
	Threshold was set improperly	Set the threshold above the noise and where the replicates are tightest. Refer to your real-time PCR system user documentation for procedures on setting the threshold.
	Low concentration of target	Rerun the assay using more template.
	Template absorption (adhering to the tube)	Add a carrier (for example, yeast tRNA).
C_T value is lower than expected.	gDNA contamination	• Perform bioinformatics: Design the assay to span an exon-exon junction. For more information, refer to the: – *Custom TaqMan® Genomics Assays Protocol: Submission Guidelines* (PN 4367671) – *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (from **www.appliedbiosystems.com**) • Verify contamination by running an RT-minus reaction (without the reverse transcriptase). • Treat the sample with DNase.
	More sample added than expected	• Reduce the amount of sample. • Quantitate and normalize the sample.
	Template or amplicon contamination	Follow established PCR good laboratory practices.
Amplification occurs in the no RT controls.	gDNA contamination	• Perform bioinformatics: Design the assay to span an exon-exon junction. For more information, refer to the: – *Custom TaqMan® Genomics Assays Protocol: Submission Guidelines* (PN 4367671) – *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (from **www.appliedbiosystems.com**) • Improve sample extraction methods to eliminate gDNA. See "Prepare the total RNA" on page 5. • Treat the sample with DNase.
	Template or amplicon contamination	Follow established PCR good laboratory practices.
Shifting R_n value during the early cycles of the PCR (cycles 0 to 5)	Fluorescence did not stabilize to the buffer conditions of the reaction mix. **Note:** This condition does not affect PCR or the final results.	• Reset the lower value of the baseline range. • Use automatic baselining.

Table 7 Troubleshooting *(continued)*

Observation	Possible cause	Recommended action
Small ΔR_n	PCR efficiency is poor.	Recheck the concentration of the reagents.
	Quantity of starting target is low (low copy number of target).	Increase the quantity of the starting target.
Noisy signal above the threshold	Evaporation	Check the seal of the optical adhesive cover for leaks.
	Empty well due to inaccurate pipetting.	• Check the calibration of the pipettes. • Pipette more than 5 µL of sample.
	Well is labeled with a detector in the plate document/ experiment, but the well is empty.	• Be sure your plate document/experiment is set up correctly. • Exclude the well and reanalyze the data.

Appendix A How to Order TaqMan® Gene Expression Assays

This appendix covers:

Step 1: Search for an inventoried or made-to-order assay

The following table summarizes the tools and methods that you can use to search for and purchase TaqMan® Gene Expression Assays from Applied Biosystems. After you select an assay to purchase, proceed to "Step 2: Order the assay" on page 22.

Search tools and methods

Note: For an explanation of assay ID nomenclature, see "About TaqMan® Gene Expression Assay IDs" on page 21.

Method	Description
Perform a quick search	1. Go to **www.appliedbiosystems.com** 2. In the "I Want to Buy" box, select **TaqMan® Gene Expression Assays**. 3. In the Gene Expression Assays, Plates & Arrays page, select **TaqMan® Gene Expression Assays**. 4. Use the orange "Start Here" box to search for assays that match your gene of interest.
Full assay search	1. Go to **www.appliedbiosystems.com** 2. Select **Products ▸ Assay Searches ▸ TaqMan® Gene Expression Assays**. 3. Search for assays that match your gene of interest. To learn more about how to order using the full assay search, refer to the *Online Ordering Guide for TaqMan® Gene Expression Assays* (PN 127MI07-05). To select the ideal assay for your research, refer to the *Online Selection Guide for TaqMan® Gene Expression Assays* (PN 127GU08-01).
GeneAssist™ Pathway Atlas	1. Go to **www4.appliedbiosystems.com/tools/pathway** 2. Follow the instructions to search through the GeneAssist™ Pathway Atlas. This tool provides access to more than 350 interactive cell signaling maps, and allows you to view gene and disease information while you order *Silencer®* siRNAs and corresponding TaqMan® Gene Expression Assays.
UMapIt Mapping tool	1. Go to **www4.appliedbiosystems.com/tools/umapit** 2. Follow the instructions to find and order TaqMan® Gene Expression Assays for validating microarray hits or for performing follow-on experiments. This tool provides an easy way to find TaqMan® assays from microarray probe IDs.

About TaqMan® Gene Expression Assay IDs

About the Assay ID Prefix

The gene expression assay ID prefix indicates the species to which the assay is designed.

Prefix	Species
At	*Arabodopsis thaliana*
Bt	*Bos taurus* (Bovine)
Ce	*Caenorhabditis elegans*
Cf	*Canis familiaris* (Canine)
Dm	*Drosophila melanogaster*
Dr	*Danio rerio* (Zebrafish)
Hs	*Homo sapiens*
Mm	*Mus musculus*
Rh	*Macaca mulatta* (Rhesus)
Rn	*Rattus norvegicus*

About the Assay ID Suffix

The gene expression assay ID suffix indicates the assay placement.

Suffix	Definition
_m	An assay whose probe spans an exon junction and does not detect genomic DNA.
_s	An assay whose primers and probes are designed within a single exon. Such assays, by definition, detect genomic DNA.
_g	An assay that may detect genomic DNA. The assay primers and probe may also be within a single exon.
_mH _sH _gH	An assay that is designed to a transcript belonging to a gene family that has high sequence homology. The assays are designed to yield a 10- to 15-C_T difference between the target gene and the gene with the closest sequence homology. This means that an assay detects the target transcript with 1000- to 30,000-fold greater discrimination (sensitivity) than the closest homologous transcript, if both transcripts are at the same copy number in a sample.
_u	An assay whose amplicon spans an exon junction, and whose probe binds completely in one of the spanned exons.
_ft	An assay designed to detect fusion transcripts that result from chromosomal translocation. One primer and the probe are on one side of the fusion transcript breakpoint, and the second primer is on the other side of the fusion transcript breakpoint. The assay does not detect gDNA.
_at	An assay that is designed to detect a synthetic RNA transcript with a unique sequence that lacks homology to current annotated biological sequences.

Note: An assay ID beginning with "Hs999999…" and ending in "_m1" identifies a TaqMan® Gene Expression Assay that amplifies a region spanning an exon junction, although the associated probe does not span the junction. For example, the exon boundary information for assay Hs99999903_m1 is 1-1, indicating that the probe targets a region within exon 1, not the exon junction itself. Although the probe binds within a single exon, the amplicon spans exons 1-2 (the forward primer and probe are in exon 1, but the reverse primer is in exon 2).

Step 2: Order the assay

After you identify a TaqMan® Gene Expression Assay of interest, you can purchase the assay as described in this section. Many of the methods that you can use to find assays (see "Search tools and methods" on page 20) also provide methods for ordering them. When you complete your order, select and order an endogenous control as explained in "Step 3: Order a candidate endogenous control assay" on page 25.

If you cannot find an assay to suit your needs, you can:

- Order a custom assay (see below).
- Reorder a custom assay (see page 24).

TaqMan® Gene Expression Assays are available in the following formulations:

TaqMan® Gene Expression Assays	No. of 20-µL reactions	Part number	Description
Inventoried (20×)‡	250	4331182	In stock
Made-to-Order (20×)	360	4351372	Manufactured when ordered
Custom (60×)	2900	4332079	
Custom (20×)	750	4332078	
	360	4331348	
TaqMan® Endogenous Controls (20×)	50 200 1000	For human, mouse, and rat controls, see page 25. For all other species, visit the Applied Biosystems web site.	• Nonprimer limited, FAM™ dye-labeled MGB probe • Primer limited, VIC® dye-labeled MGB probe • Primer limited, VIC® dye-labeled, TAMRA™ dye-quenched Probe

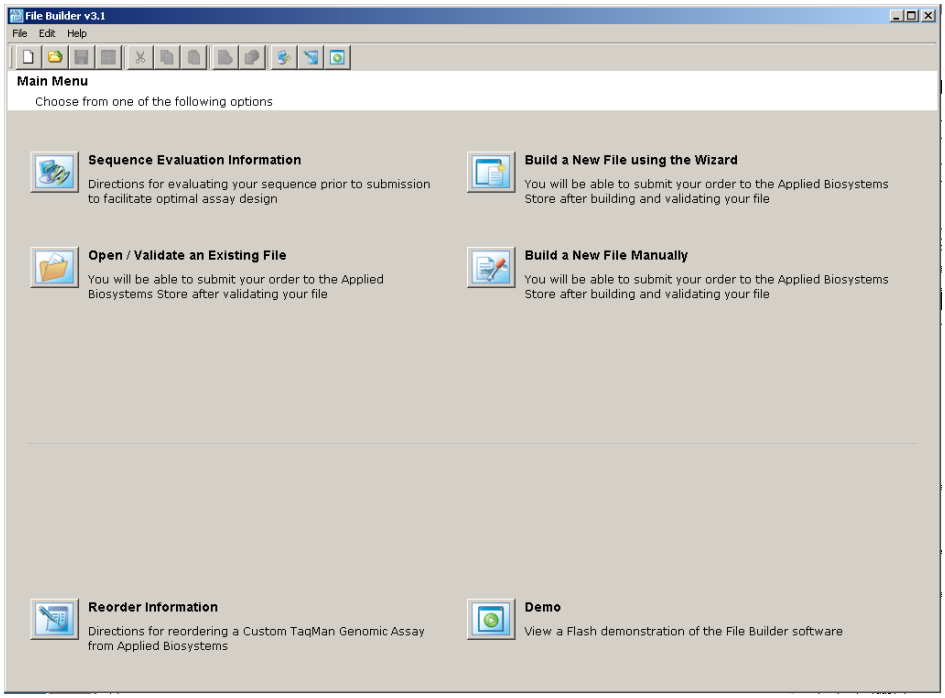
‡ TaqMan® Gene Expression Assays are preformulated at 20×.

Order a custom assay

Brief procedures for ordering new custom TaqMan® assays and for reordering previously received custom assays are described below. For details, refer to the *Custom TaqMan® Genomic Assays Protocol: Submission Guidelines* (PN 4367671). To reorder a custom assay that you purchased from Applied Biosystems, refer to "Reorder a custom assay" on page 24.

To order Custom TaqMan® Gene Expression Assays, you must create a submission file and send it to Applied Biosystems as explained below.

1. Download the File Builder software:
   a. Go to **www.appliedbiosystems.com/filebuilder**
   b. Click the appropriate software version (PC or Mac), then save the application to your computer.
2. Install the File Builder software.
3. Start the File Builder software, then select **Build a New File Using the Wizard**.



4. Create a submission file with the following information:
   - Your name
   - Your phone number
   - Your e-mail address
   - The DNA sequence name
   - The DNA sequence (>61 bases)
   - The target site coordinate, which contains the target site and target site name

5. Send the file to Applied Biosystems by one of the following methods:
   - Use the File Builder software to upload the file to the Applied Biosystems store.
   - E-mail the file to your regional Custom TaqMan® Genomic Assays sales office (see the table below). In the Subject line, enter **Custom TaqMan Genomic Assays Order Information**.

Region	E-mail address
Africa, CIS, Europe, Middle East, and West Asia	assays.europe@eurappliedbiosystems.com
Australia and New Zealand	abozorders@appliedbiosystems.com
Japan	JPOrderAdminCS@appliedbiosystems.com
North America	genomics@appliedbiosystems.com

Note: For other regional offices, visit **www.appliedbiosystems.com**

When you complete your order, select and order an endogenous control as explained in "Step 3: Order a candidate endogenous control assay" on page 25.

Reorder a custom assay

Note: To order a *new* assay, refer to "Order a custom assay" on page 22. For details on ordering custom assays, refer to the *Custom TaqMan® Genomic Assays Protocol: Submission Guidelines* (PN 4367671).

To reorder a custom assay:

1. Collect the following information:
   - Part number of the assay that you want to reorder
   - Original assay ID (on the assay tube and in the AIF that was shipped with the assay)
   - Original sales order number(s) (packing slip, AIF on the CD, and the invoice)

   Note: To ensure that you receive primers and probe sequences identical to those in your original order, Applied Biosystems uses the assay ID and sales order number to retrieve your previous assay design information.

   IMPORTANT! Do not submit the target DNA sequence unless you want to redesign your assay. New versions of Applied Biosystems design software may create assays with different primer and probe sequences, which can lead to a difference in assay performance.

2. E-mail the information as an attachment to your regional Custom TaqMan® Genomic Assays sales office (see the table below). In the Subject line, enter **Custom TaqMan Genomic Assays Reorder Information**.

Region	E-mail address
Africa, CIS, Europe, Middle East, and West Asia	**assays.europe@eurappliedbiosystems.com**
Australia and New Zealand	**abozorders@appliedbiosystems.com**
Japan	**JPOrderAdminCS@appliedbiosystems.com**
North America	**genomics@appliedbiosystems.com**

   Note: For other regional offices, visit **www.appliedbiosystems.com**

   Note: You can reorder multiple assays in a single e-mail.

When you complete your order, select and order an endogenous control as explained in "Step 3: Order a candidate endogenous control assay" on page 25.

Step 3: Order a candidate endogenous control assay

To help with normalization, you can select a human, mouse, or rat endogenous control assay from the list of inventoried endogenous controls in Tables 8 and 9.

Use Table 8 to order…	Use Table 9 on page 26 to order…
Non-primer limited, FAM™ dye-labeled MGB probe	• Non-primer limited, FAM™ dye-labeled MGB probe • Primer limited, VIC® dye-labeled MGB probe • Primer limited, VIC® dye-labeled, TAMRA™ dye-quenched probe

After you select an endogenous control for your experiment, search for and order the endogenous control assay using one of the methods described in "Search tools and methods" on page 20. After you submit your order, go to "Step 4: Select a chemistry" on page 28.

Assays for normalization

A valid normalization or endogenous control is needed to correct for differences in RNA sampling and sample variation. The ideal control is expressed consistently under experimental conditions and is sufficiently abundant across all tissues and cell types studied.

Note: Applied Biosystems recommends that you experimentally validate all candidate genes to be used as endogenous controls.

Table 8 Candidate endogenous control assays for normalization (PN 4331182)

Gene symbol	Gene name	Human assay ID	Mouse assay ID	Rat assay ID
18S	Eukaryotic 18S rRNA	Hs99999901_s1	Hs99999901_s1	Hs99999901_s1
ACTB	Actin, Beta, cytoplasmic	Hs99999903_m1	Mm00607939_s1	Rn00667896_m1
B2M	Beta-2-microglobulin	Hs99999907_m1	Mm00437762_m1	Rn00560865_m1
GAPDH	Glyceradehyde-3-phosphate dehydrogenase	Hs99999905_m1	Mm99999915_g1	Rn99999916_s1
GUSB	Beta glucuronidase	Hs99999908_m1	Mm00446953_m1	Rn00566655_m1
HMBS	Hydromethylbilane synthase	Hs00609297_m1	Mm00660262_g1	Rn00565886_m1
HPRT1	Hypoxanthine guanine phosphoribosyl transferase 1	Hs99999909_m1	Mm00446968_m1	Rn01527840_m1
IP08	Importin 8	Hs00183533_m1	Mm01255158_m1	Not Available
PGK1	Phosphoglycerate kinase 1	Hs99999906_m1	Mm00435617_m1	Rn00821429_g1
POLR2A	Polymerase (RNA) II (DNA directed) polypeptide A, 220 kDa	Hs00172187_m1	Mm00839493_m1	Rn01752026_m1
PPIA	Peptidylprolyl isomerase A	Hs99999904_m1	Mm02342430_g1	Rn00690933_m1
RPLP0	Ribosomal protein, large, P0	Hs99999902_m1	Mm00782638_s1	Rn01479927_g1
TBP	TATA box binding protein	Hs99999910_m1	Mm00446973_m1	Rn01455648_m1
TFRC	Transferrin receptor	Hs99999911_m1	Mm00441941_m1	Rn01474695_m1
UBC	Ubiquitin C	Hs00824723_m1	Mm01201237_m1	Rn01789812_g1
YWHAZ	Tyrosine 3-monooxygenase, or tryptophan 5-monooxygenase activation protein, zeta polypeptide	Hs00237047_m1	Mm01158417_g1	Rn00755072_m1

Table 9 TaqMan® Endogenous Controls

Species	Gene symbol	Gene name	Reporter dye	Quencher	Primer limited	Part number	No. of 20-µL rxns.	Corresponding TaqMan® Assay ID in Table 8 (PN 4331182)
Eukaryotic	18S	18S ribosomal RNA	FAM™	MGB	No	4333760T	125	Hs99999901_s1
			FAM™	MGB	No	4333760F	500	Hs99999901_s1
			FAM™	MGB	No	4352930E	2500	Hs99999901_s1
			VIC®	MGB	Yes	4319413E	2500	N/A
			VIC®	TAMRA™	Yes	4310893E	2500	N/A
Human	ACTB	Beta actin	FAM™	MGB	No	4333762T	125	Hs99999903_m1
			FAM™	MGB	No	4333762F	500	Hs99999903_m1
			FAM™	MGB	No	4352935E	2500	Hs99999903_m1
			VIC®	MGB	Yes	4326315E	2500	N/A
			VIC®	TAMRA™	Yes	4310881E	2500	N/A
	B2M	Beta-2-microglobulin	FAM™	MGB	No	4333766T	125	Hs99999907_m1
			FAM™	MGB	No	4333766F	500	Hs99999907_m1
			VIC®	MGB	Yes	4326319E	2500	N/A
			VIC®	TAMRA™	Yes	4310886E	2500	N/A
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	FAM™	MGB	No	4333764T	125	Hs99999905_m1
			FAM™	MGB	No	4333764F	500	Hs99999905_m1
			FAM™	MGB	No	4352934E	2500	Hs99999905_m1
			VIC®	MGB	Yes	4326317E	2500	N/A
			VIC®	TAMRA™	Yes	4310884E	2500	N/A
	GUSB	Beta glucuronidase	FAM™	MGB	No	4333767T	125	Hs99999908_m1
			FAM™	MGB	No	4333767F	500	Hs99999908_m1
			VIC®	MGB	Yes	4326320E	2500	N/A
			VIC®	TAMRA™	Yes	4310888E	2500	N/A
	HPRT1	Hypoxanthine-phosphoribosyl transferase 1	FAM™	MGB	No	4333768T	125	Hs99999909_m1
			FAM™	MGB	No	4333768F	500	Hs99999909_m1
			VIC®	MGB	Yes	4326321E	2500	N/A
			VIC®	TAMRA™	Yes	4310890E	2500	N/A
	PGK1	Phosphoglycerate kinase 1	FAM™	MGB	No	4333765T	125	Hs99999906_m1
			FAM™	MGB	No	4333765F	500	Hs99999906_m1
			VIC®	MGB	Yes	4326318E	2500	N/A
			VIC®	TAMRA™	Yes	4310885E	2500	N/A
	PPIA	Cyclophilin A	FAM™	MGB	No	4333763T	125	Hs99999904_m1
			FAM™	MGB	No	4333763F	500	Hs99999904_m1
			VIC®	MGB	Yes	4326316E	2500	N/A
			VIC®	TAMRA™	Yes	4310883E	2500	N/A

Table 9 TaqMan® Endogenous Controls *(continued)*

Species	Gene symbol	Gene name	Reporter dye	Quencher	Primer limited	Part number	No. of 20-µL rxns.	Corresponding TaqMan® Assay ID in Table 8 (PN 4331182)
Human	RPLP0	Ribosomal protein, large, P0	FAM™	MGB	No	4333761T	125	Hs99999902_m1
			FAM™	MGB	No	4333761F	500	Hs99999902_m1
			VIC®	MGB	Yes	4326314E	2500	N/A
			VIC®	TAMRA™	Yes	4310879E	2500	N/A
	TBP	TATA-box binding protein	FAM™	MGB	No	4333769T	125	Hs99999910_m1
			FAM™	MGB	No	4333769F	500	Hs99999910_m1
			VIC®	MGB	Yes	4326322E	2500	N/A
			VIC®	TAMRA™	Yes	4310891E	2500	N/A
	TFRC	Transferrin receptor (p90, CD71)	FAM™	MGB	No	4333770T	125	Hs99999911_m1
			FAM™	MGB	No	4333770F	500	Hs99999911_m1
			VIC®	MGB	Yes	4326323E	2500	N/A
			VIC®	TAMRA™	Yes	4310892E	2500	N/A
Mouse	ACTB	Beta actin	FAM™	MGB	No	4352933E	2500	Mm00607939_s1
			VIC®	MGB	Yes	4352341E	2500	N/A
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	FAM™	MGB	No	4352932E	2500	Mm99999915_g1
			VIC®	MGB	Yes	4352339E	2500	N/A
Rat	ACTB	Beta actin	FAM™	MGB	No	4352931E	2500	Rn00667869_m1
			VIC®	MGB	Yes	4352340E	2500	N/A
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	FAM™	MGB	No	4352936E	2500	Rn99999916_s1
			VIC®	MGB	Yes	4352338E	2500	N/A

Step 4: Select a chemistry

Select standard or Fast chemistry

StepOne™, StepOnePlus™, 7500 Fast, and 7900HT Fast Real-Time PCR Systems contain Fast thermal cycling blocks that can perform Fast quantitative PCR. Applied Biosystems Fast PCR systems use high-speed thermal cycling blocks, TaqMan® Fast Universal PCR Master Mix, and optical Fast thermal cycling plates and tubes to reduce quantitative PCR run times to less than 40 minutes. For more information on Fast chemistries available from Applied Biosystems, refer to the Data Sheet: *Comparing Fast and Standard Data on Applied Biosystems 7500 and 7500 Fast Real-Time PCR Systems* (SN 117MI08-01).

The following table indicates the chemistry and plates for a standard or Fast run.

Component	Standard chemistry per run	Fast chemistry per run
cDNA quantity	1–100 ng	1–100 ng
TaqMan® Master Mix	• TaqMan® Gene Expression Master Mix • TaqMan® Universal PCR Master Mix (2×), with or without AmpErase® UNG	TaqMan® Fast Universal PCR Master Mix (2×), No AmpErase® UNG
96-well plate	MicroAmp® Optical 96-Well Reaction Plate with Barcode	MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode
384-well plate	MicroAmp® Optical 384-Well Reaction Plate with Barcode	
48-well plate	MicroAmp® Fast Optical 48-Well Reaction Plate	

Select 1- or 2-step PCR

Applied Biosystems offers several chemistries that you can use to perform RT-PCR in 1- or 2-steps. See "Step 5: Order materials and equipment not included" on page 29 for a list of kits.

Step 5: Order materials and equipment not included

After you order the target and endogenous control assays, order the reagents (Table 10) and consumables (Table 11 on page 30) required for the experiment (see "Materials and equipment not included" on page 3 for a list of required materials).

To order…	See…
Related reagents	Table 10
Reaction plates and accessories	Table 11 on page 30
Related gene expression assays and arrays products	Table 12 on page 32

Related reagents

Table 10 Reagents for reverse transcription and PCR

Reagent	Description and part number
TaqMan® Gene Expression Master Mix (2X)	• One 1-mL tube (PN 4370048) • One 5-mL bottle (PN 4369016) • One 6-mL bottle (PN 4393469) • Two 5-mL bottles (PN 4369514) • Five 5-mL bottles (PN 4369510) • Ten 5-mL bottles (PN 4369542) • One 50-mL bottle (PN 4370074)
TaqMan® Universal PCR Master Mix (2X)	• One 5-mL bottle (PN 4304437) • Two 5-mL bottles (PN 4364338) • Five 5-mL bottles (PN 4364340) • Ten 5-mL bottles (PN 4305719) • One 50-mL bottle (PN 4326708)
TaqMan® Universal Master Mix (2X) No AmpErase® UNG	• One 5-mL bottles (PN 4324018) • Two 5-mL bottles (PN 4364341) • Five 5-mL bottles (PN 4364343) • Ten 5-mL bottles (PN 4324020) • One 50-mL bottle (PN 4326614)
TaqMan® Fast Universal Master Mix (2X) No AmpErase® UNG	• 250 × 20-µL reactions (PN 4352042) • 500 × 20-µL reactions (PN 4366072) • 1250 × 20-µL reactions (PN 4366073) • 2500 × 20-µL reactions (PN 4364103) • 5000 × 20-µL reactions (PN 4367846)
High Capacity cDNA Reverse Transcription Kit	• 200 reactions (PN 4368814) • 200 reactions with RNase Inhibitor (PN 4374966) • 1000 reactions (PN 4368813) • 1000 reactions with RNase Inhibitor (PN 4374967)
TaqMan® RNA-to-C_T™ *1-Step* Kit	• 40 × 50-µL reactions (PN 4392653) • 200 × 50-µL reactions (PN 4392938) • 2000 × 50-µL reactions (PN 4392656)
Nuclease-free water (no diethyl pyrocarbonate [DEPC])	500 mL (PN AM9930)

Reaction plates and accessories

Table 11 Reaction plates and accessories for Applied Biosystems thermal cyclers and real-time PCR systems

Instrument	Reaction plates and accessories
7300 system	• MicroAmp® Optical 96-Well Reaction Plate with Barcode: – 500 plates (PN 4326659) – 20 plates (PN 4306737) • MicroAmp® Optical Adhesive Film (PN 4311971) • MicroAmp® Optical Film Compression Pad (PN 4312639) • MicroAmp® Optical 8-Tube Strips, 0.2-mL, 1000 tubes in strips of eight (PN 4316567) • MicroAmp® Optical 8-Cap Strips, 300 strips (PN 4323032)
7500 system	
7500 Fast system	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp® Optical Adhesive Film (PN 4311971)
7900HT Fast system, standard 96-well block	• MicroAmp® Optical 96-Well Reaction Plate with Barcode: – 500 plates (PN 4326659) – 20 plates (PN 4306737) • MicroAmp® Optical Adhesive Film (PN 4311971) • MicroAmp® Optical Film Compression Pad (PN 4312639) for use with one plate
7900HT Fast system, Fast 96-well block	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp® Optical Adhesive Film (PN 4311971) • MicroAmp® Optical Film Compression Pad (PN 4312639) for use with one plate
7900HT Fast system, 384-well block	• MicroAmp® Optical 384-Well Reaction Plate with Barcode: – 1000 plates (PN 4343814) – 500 plates (PN 4326270) – 50 plates (PN 4309849) • MicroAmp® Optical 384-Well Reaction Plate, 1000 plates (PN 4343370) • MicroAmp® Optical Adhesive Film (PN 4311971)
StepOne™ system	• MicroAmp® Fast Optical 48-Well Reaction Plate, 20 plates (PN 4375816) • MicroAmp® 48-Well Optical Adhesive Film (PN 4375323)
StepOnePlus™ system	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: – 200 plates (PN 4366932) – 20 plates (PN 4346906) • MicroAmp® Optical Adhesive Film (PN 4311971)

Table 11 Reaction plates and accessories for Applied Biosystems thermal cyclers and real-time PCR systems *(continued)*

Instrument	Reaction plates and accessories
9700 instrument	• MicroAmp® Optical 96-Well Reaction Plate with Barcode: – 500 plates (PN 4326659) – 20 plates (PN 4306737) • ABI PRISM® 384-Well Clear Optical Reaction Plate with Barcode: – 1000 plates (PN 4343814) – 500 plates (PN 4326270) – 50 plates (PN 4309849) • MicroAmp® Optical Adhesive Film (PN 4311971) • MicroAmp® Clear Adhesive Films, 100 films (PN 4306311) • MicroAmp® Optical 8-Tube Strips, 0.2-mL, 1000 tubes in strips of eight (PN 4316567) • MicroAmp® Optical 8-Cap Strips, 300 strips (PN 4323032)
Veriti™ 96-well thermal cycler	• MicroAmp™ Optical 96-Well Reaction Plate: – 500 plates (PN 4316813) – 10 plates (PN N8010560) • MicroAmp® Optical Adhesive Film (PN 4311971) • MicroAmp® Clear Adhesive Films, 100 films (PN 4306311)
Veriti™ 384-well thermal cycler	• MicroAmp™ Optical 384-Well Reaction Plate with Barcode: – 1000 plates (PN 4343814) – 500 plates (PN 4326270) – 50 plates (PN 4309849) • MicroAmp® Optical Adhesive Film (PN 4311971)

Related gene expression assays and arrays products

Table 12 Related gene expression assays and arrays products

Assay or array		For more information...
TaqMan® Assays	TaqMan® Express Plates‡	**www.allgenes.com**
	TaqMan® MicroRNA Assays	**miRNA.appliedbiosystems.com**
	Custom TaqMan® Small RNA Assays	Contact a Applied Biosystems Sales Representative
	Custom TaqMan® Probes and Primers§	**www.appliedbiosystems.com**
TaqMan® Arrays	TaqMan® Arrays: • TaqMan® Custom Arrays • TaqMan® Gene Signature Array • TaqMan® Gene Sets	**taqmanarray.appliedbiosystems.com**
	Megaplex™ Pools for microRNA Expression Analysis: • Megaplex™ RT Primers • Megaplex™ PreAmp Primers • TaqMan® MicroRNA Arrays	**miRNA.appliedbiosystems.com**

‡ TaqMan® Gene Expression Assays dried in MicroAmp® Optical 96-Well Reaction Plates.
§ Probes and primers synthesized by Applied Biosystems to your exact sequence and choice of quencher and reporter dyes.

Appendix B Good PCR Practices

Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

AmpErase® UNG

AmpErase® Uracil-N-glycosylase (UNG) prevents reamplification of carryover-PCR products in an assay if all previous PCR for that assay is performed using a dUTP-containing master mix. UNG acts on single- and double-stranded dU-containing DNA by hydrolyzing uracil-glycosidic bonds at dU-containing DNA sites. The enzyme causes the release of uracil, thereby creating an alkali-sensitive apyrimidic site in the DNA. The enzyme has no activity on RNA or dT-containing DNA (Longo *et al.*, 1990).

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

Appendix C Background Information

This appendix covers:

About TaqMan® chemistry

About the probes

The TaqMan® MGB probes contain:

- A reporter dye (for example, FAM™ dye) linked to the 5′ end of the probe
- A minor groove binder (MGB) at the 3′ end of the probe

  MGBs increase the melting temperature (T_m) without increasing probe length (Afonina *et al.,* 1997; Kutyavin *et al.,* 1997); they also allow for the design of shorter probes.
- A nonfluorescent quencher (NFQ) at the 3′ end of the probe

  Because the quencher does not fluoresce, Applied Biosystems real-time PCR systems can measure reporter dye contributions more accurately.

About the 5′ nuclease assay

The 5′ nuclease assay process (Figures 3 through 6) takes place during PCR amplification. This process occurs in every cycle and does not interfere with the exponential accumulation of product.

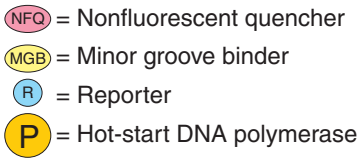


Figure 2 Legend for Figures 3 through 6

During PCR, the TaqMan® MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites (Figure 3).

When the probe is intact (Figures 3 and 4), the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer (Förster, 1948; Lakowicz, 1983).

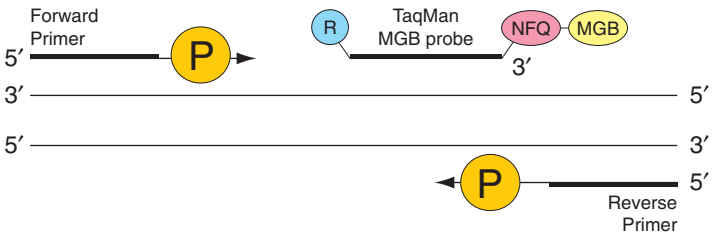


Figure 3 Polymerization

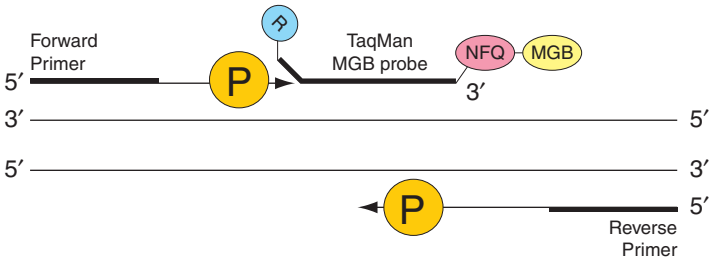


Figure 4 Strand displacement

The DNA polymerase cleaves only probes that are hybridized to the target (Figure 5). Cleavage separates the reporter dye from the quencher dye; the separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter. The increase in fluorescence occurs only if the target sequence is complementary to the probe and is amplified during PCR. Because of these requirements, nonspecific amplification is not detected.

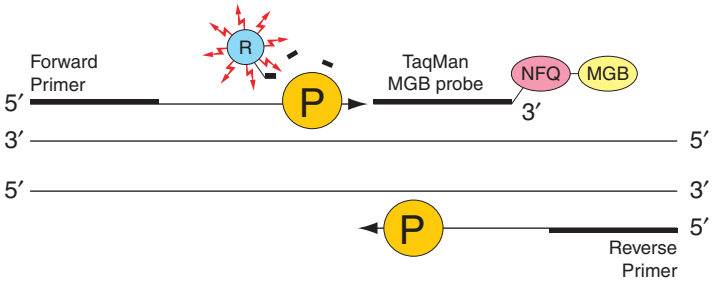


Figure 5 Cleavage

Polymerization of the strand continues, but because the 3′ end of the probe is blocked, there is no extension of the probe occurs during PCR (Figure 5).

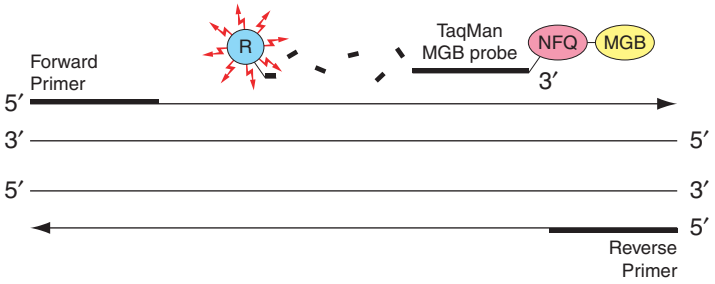


Figure 6 Completion of polymerization

About the TaqMan® Gene Expression Assays Information CD

When you order TaqMan® Gene Expression Assays, you receive an Information CD with your order that contains:

- An Assay Information File (AIF): *ProdNum_LotNum*_AIF.txt
  where, *ProdNum* is the manufacturing production number and *LotNum* is the lot number of the assay. More than one lot number may be associated with one production number.
- *TaqMan® Gene Expression Assays Protocol* (PN 4333458)
- *TaqMan® Gene Expression Assays Quick Reference Card* (PN 4401212)

About the assay information file (AIF)

The Assay Information File (AIF) is a text file that describes the TaqMan® Gene Expression Assay.

To view the assay information file as a spreadsheet in Microsoft Excel:

1. Load the TaqMan® Gene Expression Assay Information CD into the CD drive.
2. Navigate to the drive that contains the Information CD.
3. Click, then right-click ***ProdNum_LotNum*_AIF.txt**, then select **Open with Excel**.

AIF columns

Table 13 describes the columns of the AIF.

Note: In Table 13, "blank" appears in the Example column for fields that do not apply to TaqMan® Gene Expression Assays or Custom TaqMan® Gene Expression Assays.

Table 13 Fields of the assay information file

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	Custom TaqMan® Gene Expression Assays
Customer Name	Your organization or institution	Company XYZ	Company XYZ
(Sales) Order Number	A unique number that identifies the Applied Biosystems sales order	1234567890	1234567890
Ship Date	The date when the assay was packaged for shipment	7-Nov-2008	7-Nov-2008
Delivery Number (Shipment ID)	A unique bar code number that identifies the shipment **Note:** The shipment ID also appears in the plate ID.	880309546	880309546
Part Number	A number that identifies the product line	4351372	4331348
Product Type	The Applied Biosystems product line associated with the assay	TaqMan® Gene Expression Assays	Custom TaqMan® Gene Expression Assays
Assay ID	An alphanumeric string that identifies the assay	Rn01648213_m1	KR14TD-A22T
Lot Number	A unique alphanumeric string that identifies the manufacturing batch to which the assay belongs	080227T100615	080227T100615

Table 13 Fields of the assay information file *(continued)*

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	Custom TaqMan® Gene Expression Assays
Shipping Rack or Plate Type	The type of container in which the assay is shipped (such as a 96-position or a 16-position tube rack)	96-position tube rack v1	96-position tube rack v1
Shipping Rack or Plate ID	A bar code number on the label of each shipped rack or plate that consists of the shipment ID plus a unique numeric suffix that identifies the rack or plate containing the assay.	880309546-1	880309546-1
Vial/Tube Type	The type of vial or tube that contains the assay	2D barcode labeled tube	2D barcode labeled tube
Vial/Tube ID	A unique, 10-digit bar code number on the bottom of each assay vial or tube that identifies it	0004696076	0004696076
Well Location on the Shipping Rack or Plate	The location of the assay on the associated shipping rack or plate	B02	B02
Assay Mix Concentration	The concentration of the assay, including both primers and probe	20×	20×
Forward Primer Name	The name of the forward primer, assigned by the design software, that consists of the assay ID plus an “F” suffix	(blank)	KR14TD-A22TF
Forward Primer Sequence	The nucleotide sequence of the forward primer	(blank)	GGACTTGCACGACTAA
Forward Primer Concentration	The concentration of the forward primer (µM)	18	18
Reverse Primer Name	The name of the reverse primer, assigned by the design software, that consists of the assay ID plus an “R” suffix	(blank)	KR14TD-A22TR
Reverse Primer Sequence	The nucleotide sequence of the reverse primer	(blank)	CCGTACGTCAATTGAC
Reverse Primer Concentration	The concentration of the reverse primer (µM)	18	18
Reporter 1 Name	The name of the reporter 1 oligonucleotide probe, assigned by the design software, that consists of the assay ID and a suffix code (M1 or M2). The letter in the suffix code identifies the reporter dye that is covalently bound to the fluorogenic probe. The number identifies the DNA strand used to design the probe: • 1 – Forward strand design • 2 – Reverse strand design For example, in the name “KR14TD-A22TM1,” the letter “M” indicates that the probe is labeled with the FAM™ dye, and the number “1” indicates that the probe was designed to the forward strand.	(blank)	KR14TD-A22TM1
Reporter 1 Dye	The reporter dye label for the reporter 1 probe	FAM™	FAM™
Reporter 1 Sequence	The nucleotide sequence of the reporter 1 probe	(blank)	TTCGAACTGATCAT
Reporter 1 Concentration	The concentration of the reporter 1 probe (µM)	5	5

Table 13 Fields of the assay information file *(continued)*

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	Custom TaqMan® Gene Expression Assays
Reporter 1 Quencher	The quencher used for reporter 1 probe (for example, Minor Groove Binder-Non Fluorescing Quencher [MGB-NFQ])	MGB-NFQ	MGB-NFQ
Reporter 2 Name	Not applicable to TaqMan® Gene Expression Assay.	(blank)	(blank)
Reporter 2 Dye			
Reporter 2 Sequence			
Reporter 2 Concentration			
Reporter 2 Quencher			
Context Sequence	The 25-nucleotide sequence surrounding the probe, including the targeted exon(s)	...NNNNNNNNN...	(blank)
Design Strand	Indicates the strand used to design the probe: • Forward – The probe binds to the same strand as the forward primer. • Reverse – The probe binds to the same strand as the reverse primer.	(blank)	Forward
Category	The Celera Panther Protein Classification (Level 1) for the gene	Chromosome 9	(blank)
Category ID	A unique, 10-character alphanumeric abbreviation of the Panther category classification for the assay	Chr9	(blank)
Group	The Celera Panther Protein Classification (Level 2) for the gene	D9S1776-D9S1682	(blank)
Group ID	A unique, 10-character alphanumeric abbreviation of the Panther group classification for the assay	D9S1776	(blank)
Gene Symbol	The Entrez Gene symbol for the gene	SLC25A14	(blank)
Gene Name	The Entrez Gene name for the gene	solute carrier family 25 (mitochondrial carrier, brain), member 14	(blank)
Chromosome	The chromosome containing the gene	9	(blank)
Species	The organism for which the assay was designed	Homo_sapiens	(blank)
Target Exons	The public accession number(s) of the exon(s) that are spanned by the probe	2	(blank)
NCBI Gene Reference	The NCBI transcript identification number that corresponds to the gene	NM_001735	(blank)
NCBI SNP Reference	Not applicable to TaqMan® Gene Expression Assays.	(blank)	(blank)
Medline Reference	PubMed references for the gene	(blank)	(blank)
Celera ID	The unique Celera Discovery System (CDS) assay identification number for the gene	hCV11720402	(blank)

Table 13 Fields of the assay information file *(continued)*

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	**Custom TaqMan® Gene Expression Assays**
Cytogenetic Band	The chromosomal band where the gene is located. If unavailable, then the chromosome number is provided.	9q34	(blank)
SNP Type	Not applicable to TaqMan® Gene Expression Assay.	(blank)	(blank)
Minor Allele Frequency - Caucasian			
Minor Allele Frequency -African-American			
Minor Allele Frequency -Japanese			
Minor Allele Frequency -Chinese			
Celera Assembly Build Number			
Location on Celera Assembly			
NCBI Assembly Build Number			
Location on NCBI Assembly			

Appendix D Safety

This appendix covers:

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 45.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you select

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

WARNING! HAZARDOUS WASTE. Refer to Material Safety Data Sheets (MSDSs) and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

  IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at: **www.cdc.gov**

Chemical alerts

General alerts for all chemicals

Avoid contact with (skin, eyes, and/or clothing). Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts

CAUTION! CHEMICAL HAZARD. TaqMan® Gene Expression Master Mix, TaqMan® Universal PCR Master Mix (2×) with or without AmpErase® UNG, TaqMan® Fast Universal PCR Master Mix (2×), No AmpErase® UNG may cause eye and skin irritation.

Afonina, I., Zivarts, M., Kutyavin, I.*, et al.,* 1997. Efficient priming of PCR with short oligonucleotides conjugated to a minor groove binder. *Nucleic Acids Res.* 25:2657–2660.

Förster, V. T. 1948. Zwischenmolekulare Energiewanderung und Fluoreszenz. *Annals of Physics* (Leipzig) 2:55–75.

Kutyavin, I.V., Lukhtanov, E.A., Gamper, H.B., and Meyer, R.B. 1997. Oligonucleotides with conjugated dihydropyrroloindole tripeptides: base composition and backbone effects on hybridization. *Nucleic Acids Res.* 25:3718–3723.

Lakowicz, J.R. 1983. *Energy Transfer. In Principles of Fluorescence Spectroscopy*, New York: Plenum Press 303–339.

Longo, M.C., Berninger, M.S., and Hartley, J.L. 1990. Use of uracil DNA glycosylase to control carryover contamination in polymerase chain reactions. *Gene* 93:125–128.

Related documentation

For additional documentation, see "How to obtain support" on page 52.

Real-time PCR system	Document	Part number
All real-time PCR systems	*Custom TaqMan® Genomic Assays Protocol: Submission Guidelines*	4367671
	Online Ordering Guide for TaqMan® Gene Expression Assays	127MI07-05
	Online Selection Guide for TaqMan® Gene Expression Assays	127GU08-01
	TaqMan® Gene Expression Assays Application Note: Amplification Efficiency of TaqMan® Gene Expression Assays	127AP05-03
	TaqMan® Gene Expression Assays Application Note: Using TaqMan® Endogenous Control Assays to Select an Endogenous Control for Experimental Studies	127AP08-01
	Real-Time PCR Systems Chemistry Guide	4348358
	High-Capacity cDNA Reverse Transcription Protocol	4375575
	TaqMan® Gene Expression Master Mix Protocol	4371135
	TaqMan® Universal PCR Master Mix (2X) Protocol	4304449
	TaqMan® Fast Universal PCR Master Mix (2X) Protocol	4351891
	TaqMan® RNA-to-C_T™ 1-Step Kit Protocol	4393463
7900HT Fast system Fast or standard sample blocks	*Applied Biosystems 7900HT Fast Real-Time PCR System Quick Reference Card: Performing Fast Gene Quantification*	4351892
	Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide	4364016
	Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification	4352533
7300, 7500, and 7500 Fast systems	*Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantification Getting Started Guide*	4347828
StepOne™ and StepOnePlus™ systems	*Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide*	4379704
	Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_T Experiments Getting Started Guide	4376785

Portable document format (PDF) versions of this guide and the documents listed above are also available at **www.appliedbiosystems.com**

Note: To open the documentation available from the Applied Biosystems web site, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating only to documentation. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com** as explained above.

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