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TaqMan® Gene Expression Assays

Protocol

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About This Guide

Purpose

This TaqMan® Gene Expression Assays Protocol provides instructions for performing real-time reverse transcription-PCR (real-time RT-PCR) using TaqMan Gene Expression Assays and TaqMan Non-coding RNA Assays.

Safety information

Note: For general safety information, see this section and Appendix E, "Safety" on page 59. When a hazard symbol and hazard type appear by an instrument hazard, see the "Safety" Appendix for the complete alert on the instrument.

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.

CAUTION! – 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.

DANGER! – 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.

SDSs

The SDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 61.

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

TaqMan® Gene Expression Assays

Product information

Purpose of the product

Applied Biosystems offers comprehensive collections of predesigned, preformulated primer and probe sets that help researchers perform quantitative gene expression studies on a variety of species.

- **TaqMan® Gene Expression Assays** – Target protein-coding transcripts from a variety of species, including human, mouse, rat, *Arabidopsis*, *C. elegans*, and *Drosophila*. See Table 6 on page 33 for a complete list of species.
- **TaqMan®** Non-coding RNA Assays – Target long non-coding RNA (ncRNA) in human, mouse, and rat species. These assays are designed to ncRNAs that are >60 nt in length.

This protocol provides instructions for real-time reverse transcription-PCR (real-time RT-PCR) using TaqMan Gene Expression Assays and TaqMan Non-coding RNA Assays. Both assays are compatible with the same instruments and master mixes, and real-time RT-PCR is performed using the same procedure. Unless explicitly stated otherwise, the term "TaqMan Gene Expression Assays" is used throughout this guide to mean either assay type.

For information about TaqMan assay reactions, refer to "About TaqMan® chemistry" on page 51.

Assay formulations

TaqMan Gene Expression Assays are available as:

- **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 or TaqMan Non-coding RNA Assays. For more information on selecting endogenous controls, see "Step 3: Order a candidate endogenous control assay" on page 36.

TaqMan Non-coding RNA Assays are available as **Made-to-Order Assays**.

Kit contents

TaqMan Gene Expression Assays and TaqMan Non-coding RNA 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)
  - (TaqMan Gene Expression Assays only) One 6-VIC® dye-labeled TaqMan MGB probe (1X concentration is 250nM; 20X stock concentration is 5 µM); also available as primer limited (1X final concentration is 150 nM per primer; 20X stock concentration is 3 µM per primer)

  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 33 for more information.

- A data sheet containing information about the assay.
- An Information CD that includes the following files:
  - Assay information file (AIF)
  - *TaqMan® Gene Expression Assays Protocol* (PN 4333458)
  - *TaqMan® Gene Expression Assays Quick Reference Card* (PN 4401212)
  - *Understanding Your Shipment*, included with TaqMan Non-coding RNA Assays and certain TaqMan Gene Expression Assays

Table 1 TaqMan® Assay formulations

Product	Type	20-µL rxns.	Part number	Availability
TaqMan® Gene Expression Assays (FAM™ dye-labeled MGB probe)	Extra Small	75	4453320	Inventoried (20X)
	Extra Small	75	4448892	Made-to-Order (20×)
	Small	250	4331182	Inventoried (20×)
	Small	360	4351372	Made-to-Order (20×)
	Medium	750	4351370	
	Large	2900	4351368	Made-to-Order (60X)
TaqMan® Gene Expression Assays (VIC® dye-labeled MGB probe)	Small	360	4448489	Made-to-Order (20×)
	Medium	750	4448490	
	Large	2900	4448491	Made-to-Order (60×)
TaqMan® Gene Expression Assays Primer-Limited (VIC® dye-labeled MGB probe)	Small	360	4448484	Made-to-Order (20×)
	Medium	750	4448485	
	Large	2900	4448486	Made-to-Order (60×)

Product	Type	20-µL rxns.	Part number	Availability
TaqMan® Non-coding RNA Assay	Small	360	4426961	Made-to-Order (20X)
	Medium	750	4426962	
	Large	2900	4426963	Made-to-Order (60X)
Custom Plus TaqMan® RNA Assay	Small	360	4441114	Custom (20X)
	Medium	750	4441117	
	Large	2900	4441118	Custom (60X)
Custom TaqMan® Gene Expression Assays	Small	360	4331348	Custom (20X)
	Medium	750	4332078	
	Large	2900	4332079	Custom (60X)
TaqMan® Endogenous Controls (20X): • Non-primer limited, FAM™ dye-labeled MGB probe • Primer limited, VIC® dye-labeled MGB probe • Primer limited, VIC® dye-labeled probe • TAMRA™ dye-quenched probe	Small	125	– ‡	Inventoried (20X)
	Medium	500		
	Large	2500		

‡ See "Step 3: Order a candidate endogenous control assay" on page 36.

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 31.

Storage and dilution

- Store TaqMan Gene Expression Assay and TaqMan Non-coding RNA Assay products at –15 to –25 °C and keep them protected from light.
- To minimize freeze-thaw cycles, consider diluting 60X assays to 20X working stocks and dividing the solutions into smaller aliquots.

Materials and equipment not included

Endogenous control assay(s)

TaqMan® Endogenous Controls are a collection of predesigned assays for candidate control genes, used to normalize for differences in sample RNA added to a reaction. For a list of endogenous control assays, refer to "Step 3: Order a candidate endogenous control assay" on page 36. 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

Materials for reverse transcription and PCR

Obtain the following materials for the reverse transcription and PCR (see "Step 5: Order materials and equipment not included" on page 41 for a complete list of materials). Unless otherwise indicated, all materials are available from major laboratory suppliers (MLS).

Table 2 Required materials and equipment

✔	Material	Source
	Reverse transcription reagents	Applied Biosystems (See Table 11 on page 41)
	PCR reagents	
	Thermal cycler (or real-time PCR instrument)	Applied Biosystems
	Real-time PCR instrument	
	Reaction plates and accessories for your real-time PCR instrument	Applied Biosystems (See Table 12 on page 42)
	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

Compatible real-time instruments

TaqMan Gene Expression Assays and TaqMan Non-coding RNA 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
- ViiA™ 7 Real-Time PCR System

Workflow

Prepare the cDNA sample

Prepare the total RNA

↓

Perform reverse transcription

↓

Evaluate the cDNA

↓

High Capacity RNA-to-cDNA Kit

Thermal cycler or
real-time PCR instrument

Prepare the reaction mix and load the plate

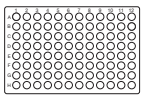
Prepare the PCR reaction mix

↓

Load the plate

↓

TaqMan® Gene Expression
Master Mix



48-, 96-, or 384-well
reaction plate

Run the real-time PCR reaction

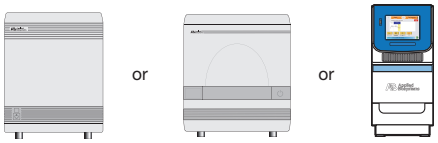
Create the plate document/experiment

↓

Run the plate

↓

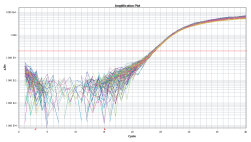
System software and information CD



Real-time PCR instrument

Analyze the results

Refer to the user guide for your real-time PCR instrument



Amplification plot

Prepare the cDNA sample

Isolate total RNA

Before running the TaqMan Gene Expression Assays, isolate total RNA to use as a template for synthesis of single-stranded cDNA. For optimal performance, Applied Biosystems recommends using an Ambion® RNA isolation kit. Go to **www4.appliedbiosystems.com**, select **RNA Isolation ▸ Which RNA Isolation Kit to Choose?** to view a list of kits.

Applied Biosystems recommends using total RNA that is:

- Between 0.002 and 0.2 µg/µL
- Less than 0.005% of genomic DNA by weight

  IMPORTANT! Assays designed to a single exon (assay IDs with _s and _g suffixes) will detect genomic DNA. When using these types of assays, if your RNA purification method does not include DNase treatment, treat the purified RNA with the Ambion® TURBO DNA-*free*™ Kit (recommended; PN AM1907) using the standard protocol.

- 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 using one of the following kits to obtain cDNA from RNA samples.

- High Capacity RNA-to-cDNA Kit (PN 4387406)
- High Capacity cDNA Reverse Transcription Kit (PN 4368813, 4374966)

Note: Use the same reverse transcription procedure for all samples in an experimental study. See Table 11 on page 41 for a list of compatible reverse transcription kits.

Evaluate the cDNA

Applied Biosystems recommends that you use:

- 1 to 100 ng of cDNA per 20-µL amplification reaction (PCR)
- The same amount of cDNA in each reaction

DNA quantitation methods

Applied Biosystems recommends that you use:

- TaqMan® RNase P Detection Reagents (recommended; PN 4316831). These reagents enable quantitation of cDNA that is able to function as a template in PCR. Refer to *Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR* (search for PN 4371090 at **www.appliedbiosystems.com**).

  or

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

(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, store the cDNA in smaller aliquots.

Prepare the reaction mix and load the plate

Thaw and mix the reagents

1. Thaw on ice, completely resuspend by gently vortexing, then briefly centrifuge to bring liquid to the bottom of the tube:
   - TaqMan Gene Expression Assays (20X)
   - cDNA samples
2. Mix the master mix reagent by gently swirling the bottle (see Table 11 on page 41 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 TaqMan 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 duplex reactions using VIC dye-labeled, primer-limited assays, see Appendix C, "Duplex Reactions" on page 49.

1. For each sample (to be run in quadruplicate), pipet the following into a nuclease-free 1.5-mL microcentrifuge tube:

PCR reaction mix component	Volume per 20-µL reaction (µL)	
	Single reaction	Four replicates#
20X TaqMan® Gene Expression Assay	1.0	5.0
2X TaqMan® Gene Expression Master Mix‡	10.0	50.0
cDNA template (1 to 100 ng)§	4.0	20.0
RNase-free water	5.0	25.0

‡ (Optional) Use TaqMan® Fast Advanced Master Mix or TaqMan® Universal Master Mix. If you add AmpErase® UNG (uracil-N-glycosylase), the final concentration must be 0.01 U/µL. Reduce the volume of water in the PCR reaction mix to compensate for additional volume from the UNG.

§ Applied Biosystems recommends that no more than 20% of the PCR be composed of the reverse transcription reaction.

Replicate volumes include 20% excess to compensate for volume loss from pipetting.

2. Cap the tube and invert it several times to mix the reaction components.
3. Centrifuge the tube briefly.

Load the plate

1. Transfer 20 µL of PCR reaction mix into each well of a 48-, 96-, or 384-well reaction plate.

   See Table 12 on page 42 for a list of compatible reaction plates and accessories.
2. Seal the plate with the appropriate cover.
3. Centrifuge the plate briefly.
4. Load the plate into the instrument.

Run the real-time PCR reaction

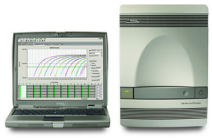
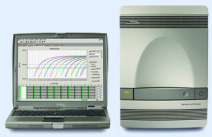
Create the plate document/experiment and run the plate

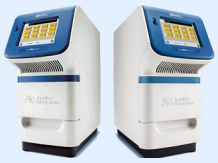
1. Create a plate document/experiment for the run using the parameter values shown in Table 3.

2. Run the plate.

   Instructions on how to create and run a plate document/experiment are on page 67 in "Related documentation", which provides a list of resource documents for your instrument.

Table 3 Plate document/experiment parameters for TaqMan® Gene Expression Assays

System	Run	Reaction plate	Plate document/ experiment parameters	Thermal cycling conditions		
				Stage	Temp (°C)	Time (mm:ss)
Applied Biosystems 7300/7500 Real-Time PCR System 	Standard	96-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard‡	Hold§	50	2:00
				Hold	95	10:00
				Cycle (40 Cycles)	95	0:15
					60	1:00
Applied Biosystems 7500 Fast Real-Time PCR System 	Standard	96-well Fast	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Hold§	50	2:00
				Hold	95	10:00
				Cycle (40 Cycles)	95	0:15
					60	1:00
	Fast	96-well Fast	• Rxn. Volume: 20 µL • Ramp Rate: Fast	Hold§	50	2:00
				Hold	95	0:20
				Cycle (40 Cycles)	95	0:03
					60	0:30
Applied Biosystems 7900HT Real-Time PCR System 	Standard	96-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Hold§	50	2:00
				Hold	95	10:00
		384-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Cycle (40 Cycles)	95	0:15
					60	1:00
	Fast	96-well Fast	• Rxn. Volume: 20 µL • Ramp Rate: Fast	Hold§	50	2:00
				Hold	95	0:20
		384-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Fast	Cycle (40 Cycles)	95	0:01
					60	0:20

System	Run	Reaction plate	Plate document/ experiment parameters	Thermal cycling conditions		
				Stage	Temp (°C)	Time (mm:ss)
Applied Biosystems StepOne™/ StepOnePlus™ Real-Time PCR System 	Standard	48-/96-well Fast	• Rxn. Volume: 20 µL • Ramp Speed: Standard	Hold§	50	2:00
				Hold	95	10:00
				Cycle	95	0:15
				(40 Cycles)	60	1:00
	Fast	48-/96-well Fast	• Rxn. Volume: 20 µL • Ramp Speed: Fast	Hold§	50	2:00
				Hold	95	0:20
				Cycle	95	0:01
				(40 Cycles)	60	0:20
Applied Biosystems ViiA™ 7 Real-Time PCR System 	Standard	384-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Hold§	50	2:00
				Hold	95	10:00
				Cycle	95	0:15
				(40 Cycles)	60	1:00
	Fast	384-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Fast	Hold§	50	2:00
				Hold	95	0:20
				Cycle	95	0:01
				(40 Cycles)	60	0:20

‡ The 7300 system has only one run mode (Standard 7300).
§ Required for optimal 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.

Resources for data analysis

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

Table 4 Data analysis guides for Applied Biosystems real-time PCR systems

Real-time PCR system	Document	Part number
7900HT Fast system	*Relative Quantitation Using Comparative C_T Getting Started Guide*	4364016
	Performing Fast Gene Quantification: Quick Reference Card	4351892
	Performing Fast Gene Quantitation with 384-Well Plates: User Bulletin	4369584
7300/7500/ 7500 Fast system	*Relative Quantification: Getting Started Guide*	4347824
	Relative Standard Curve and Comparative CT Experiments Getting Started Guide	4387783
StepOne™/ StepOnePlus™ system	*Comparative CT/Relative Standard Curve and Comparative CT Experiments Getting Started Guide*	4376785
All	*Applied Biosystems 7900HT Fast Real-Time PCR Systems and 7300/7500/7500 Fast Real-Time PCR Systems Chemistry Guide*	4348358

Tools for data analysis

Applied Biosystems recommends the following software for analyzing data generated using TaqMan® Gene Expression Assays.

DataAssist™ Software

DataAssist™ Software is a simple, yet powerful data analysis tool for sample comparison when using the comparative C_T ($\Delta\Delta C_T$) method for calculating relative quantitation of gene expression. It contains a filtering procedure for outlier removal, various normalization methods based on single or multiple genes, and provides relative quantification analysis of gene expression through a combination of statistical analysis and interactive visualization.

DataAssist™ Software is free and can be downloaded from:

www.appliedbiosystems.com/dataassist

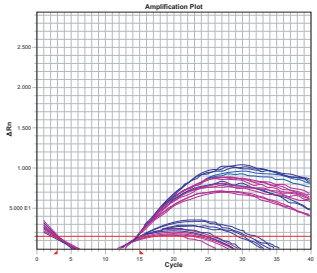
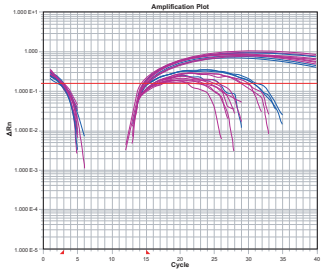
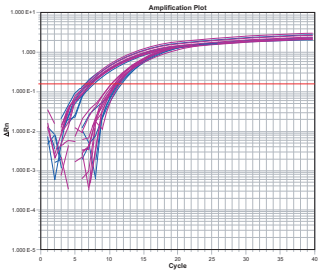
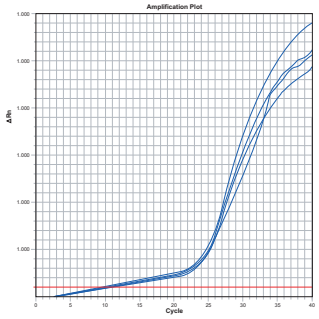
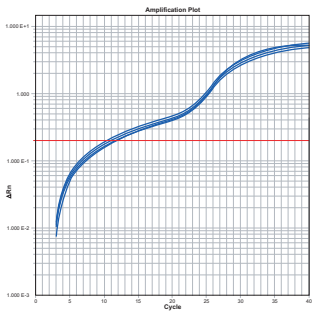
RealTime StatMiner® Software

RealTime StatMiner® Software from Integromics is a software analysis package for qPCR experiments that is compatible with all Applied Biosystems instruments. RealTime StatMiner® Software uses a step-by-step analysis workflow guide that includes parametric, non-parametric, and paired tests for relative quantification of gene expression, as well as 2-way ANOVA for two-factor differential expression analysis.

For more information, visit:

www.integromics.com/StatMiner

Troubleshooting

Observation	Possible cause	Recommended action
Amplification curve shows abnormal plot and/or low ΔRn values. Linear view:  Log view: 	The baseline was set improperly (some samples have CT 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 CT (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 CT value.
Amplification curve shows a rising baseline. Linear view:  Log view: 	There is interaction between the primer and probe.	• Adjust the threshold manually. • Select another assay from the same gene, if available.

Observation	Possible cause	Recommended action
Amplification curve shows weak amplification.	(*Custom TaqMan Gene Expression Assays only*) Sequence provided for the assay design contains mismatches with sample sequences.	Perform bioinformatics analysis. For more information, refer to the: • *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (PN 4371002). • *Custom TaqMan® Assays Design and Ordering Guide* (PN 4367671).
	Reagents and/or probe are degraded reagents.	• Check the expiration date of the reagents. • Verify that you follow the correct handling and storage conditions. • Avoid excessive freeze-thaw cycles. (Consider diluting the 60X TaqMan® Gene Expression Assay to a 20X working stock.)
	Template is contaminated or degraded.	• Improve the sample integrity (extraction methods). See "Prepare the cDNA sample" on page 15. • 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 are 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 assay for an expressed gene (for example, an endogenous control). If an inhibitor is present, low concentrations yield higher-than-expected CT values. (High concentration means more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template. 4. Improve the sample integrity (extraction methods). See "Prepare the cDNA sample" on page 15.
	The reverse transcription (RT) conversion to cDNA is poor.	• 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 the 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 occurs.	(*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.

Observation	Possible cause	Recommended action
Amplification curve shows low ROX™ dye (passive reference dye).	Inaccurate pipetting: Little or no TaqMan® Universal PCR Master Mix is present.	Follow accurate pipetting practices.
Amplification curve shows no amplification of the sample (CT = 40) across all assays or in an unusually large number of assays.	One or more of the reaction components was not added.	Verify that the cDNA, TaqMan Gene Expression Assays, 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.	Verify that 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.
	The template is degraded.	• Determine the quality of the template. • Rerun the assay with fresh template. • Use RNase-free reagents. • Use an RNase inhibitor.
	Inhibitors are 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 CT 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.
	cDNA conversion failed.	• Check the RNA integrity and concentration. • Check for RNase activity. • Follow Applied Biosystems recommended thermal profile. • Repeat the RT step using new reagents.

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (CT = 40) across all assays or in an unusually large number of assays.	(*Custom TaqMan Gene Expression Assays only*) 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.
	(*Custom TaqMan Gene Expression Assays only*) 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.
Amplification curve shows samples targeted by 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.
	Different concentrations caused by imprecise pipetting.	Follow accurate pipetting practices.
	Reagents or equipment is contaminated.	Be sure that your workspace and equipment are properly cleaned.

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (CT = 40) in the target assay.	The gene is not expressed in the tested sample.	• Verify the known expression of the gene in the sample type. • Verify by: – Rerunning the sample using the same assay. – Rerunning the assay using more sample. Avoid preparing the PCR reaction mix with more than 20% from the reverse transcription reaction. – (*TaqMan Gene Expression Assays only*) Running the sample using an alternative assay, if available, that detects a different transcript or more than one transcript from the same gene. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	The sample may not have enough copies of the target RNA.	Verify by: • Rerunning the sample using the same assay. • Rerunning the assay using more sample. Avoid preparing the PCR reaction mix with more than 20% from the reverse transcription reaction. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	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.
Decrease in ROX™ dye fluorescence (passive reference dye).	Precipitation in the TaqMan® buffers occurs.	• When using the TaqMan® PCR Core Reagents Kit, be sure to mix the tubes well. • Use TaqMan® Gene Expression Master Mix (2X). Be sure to mix thoroughly to produce a homogenous solution.
	Reagents are degraded.	Verify that kits and reagents have been stored according to the instructions on the packaging and that they have not expired.
Simultaneous increase in fluorescence from both the: • Passive reference (ROX™) dye • Reporter dye(s)	The sample evaporated.	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.
Rn on Rn-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.

Observation	Possible cause	Recommended action
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 UNG. • Run no-reverse-transcription controls to rule out genomic DNA contamination. • Treat the sample with DNase. • (*gDNA contamination only; TaqMan Gene Expression Assays only*) Design an assay that spans an exon-exon boundary.
	(*TaqMan Gene Expression Assays only*) Bacterial sequences used as template.	Use AmpliTaq Gold® LD DNA Polymerase.
The endogenous control CTs vary, or do not normalize the sample well.	Endogenous control is not consistently expressed across the samples.	Refer to the Application Note: *Using TaqMan® Endogenous Control Assays to Select an Endogenous Control for Experimental Studies* (127AP08-01) for information on selecting an endogenous control.
	Sample concentrations vary widely.	If desired, quantitate and normalize samples before running them.
	Pipetting was inaccurate.	• Check the calibration of the pipettes. • Pipet more than 5 µL of sample.
High standard deviation of replicates (inconsistent data, CT varies).	Inefficient mixing of reagents.	• Increase the length of time that you mix the reagents. • Validate your mixing process by running a replicate plate.
	Pipetting was inaccurate.	• 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 occurred (adhering to the tube).	Add a carrier (for example, yeast tRNA).

Observation	Possible cause	Recommended action
CT value is lower than expected.	gDNA contamination occurred.	• Verify contamination by running an RT-minus reaction (without the reverse transcriptase). • Treat the sample with DNase. • (*TaqMan Gene Expression Assays only*) Perform bioinformatics analysis: design a custom assay to span an exon-exon junction. For more information, refer to the: – *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (PN 4371002). – *Custom TaqMan® Assays: Design and Ordering Guide* (PN 4367671).
	More sample added than expected.	• Reduce the amount of sample. • Quantitate and adjust the concentration of the sample.
	Template or amplicon is contaminated.	Follow established PCR good laboratory practices.
Amplification occurs in the no-RT controls.	gDNA contamination occurred.	• Improve sample extraction methods to eliminate gDNA. See "Isolate total RNA" on page 15. • Treat the sample with DNase. • (*TaqMan Gene Expression Assays only)* Perform bioinformatics analysis: design a custom assay to span an exon-exon junction. For more information, refer to the: – *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (PN 4371002). – *Custom TaqMan® Assays: Design and Ordering Guide* (PN 4367671).
	Template or amplicon is contaminated.	Follow established PCR good laboratory practices.
Shifting Rn 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.
Small ΔRn.	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.

Observation	Possible cause	Recommended action
Noisy signal above the threshold.	The sample evaporated.	Check the seal of the optical adhesive cover for leaks.
	The well is empty because of inaccurate pipetting.	• Check the calibration of the pipettes. • Pipet more than 5 µL of sample.
	The well is labeled with a detector in the plate document/experiment, but the well is empty.	• Be sure that your plate document/experiment is set up correctly. • Exclude the well and reanalyze the data.

How to Order TaqMan® Gene Expression Assays

To order TaqMan® Gene Expression Assays, follow these steps:

- Step 1: Search for an assay. 32
- Step 2: Order the assay. 35
- Step 3: Order a candidate endogenous control assay 36
- Step 4: Select a chemistry. 40
- Step 5: Order materials and equipment not included. 41

Step 1: Search for an assay

Search for TaqMan® Gene Expression Assays or Non-coding RNA Assays

To perform a full assay search:

1. Go to **www.appliedbiosystems.com**.
2. Place the cursor over **Products**, then select **TaqMan Gene Expression Assays** under Assay Searches.
3. At the TaqMan Gene Expression Assays search page, select **TaqMan Gene Expression Assays**, **TaqMan Non-coding RNA Assays,** or **All Assays** from the pull-down menu.
4. Search for assays that match your target 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* (SN 127MI07-05). To select the ideal assay for your research, refer to the *Online Selection Guide for TaqMan® Gene Expression Assays* (SN 127GU08-01).

Search for TaqMan® Gene Expression Assays only

Table 5 Search tools and methods for TaqMan® Gene Expression Assays

Method	Description
Perform a quick search	1. Go to **www.appliedbiosystems.com**. 2. Under "I Want to Buy," select **TaqMan Gene Expression Assays, Plates & Arrays**. 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.
GeneAssist™ Pathway Atlas	Go to **www4.appliedbiosystems.com/tools/pathway**, then follow the instructions to search the GeneAssist™ Pathway Atlas. This tool provides access to more than 350 interactive cell signaling maps, and enables you to view gene and disease information while you order *Silencer*® siRNAs and corresponding TaqMan® Gene Expression Assays.
UMapIt™ Mapping Tool	Go to **www4.appliedbiosystems.com/tools/umapit**, then follow the instructions to find and order TaqMan® Gene Expression Assays for validating microarray hits or for performing follow-up experiments. This tool provides an easy way to find TaqMan® assays from microarray probe IDs.

About TaqMan® Gene Expression Assay IDs

Note: The assay ID prefix and suffix codes for TaqMan Gene Expression Assays and TaqMan Non-coding RNA Assays are identical.

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

Table 6 Assay ID prefix codes

Prefix	Species
At	*Arabodopsis thaliana*
Bt	*Bos taurus* (Cow)
Ce	*Caenorhabditis elegans*
Cf	*Canis familiaris* (Dog)
Cp	*Cavia. porcellus* (Guinea Pig)
Dm	*Drosophila melanogaster*
Dr	*Danio rerio* (Zebrafish)
Ec	*Equus caballus* (Horse)
Gg	*Gallus gallus* (Chicken)
Gm	*Glycine max* (Soybean)
Hs	*Homo sapiens*
Mm	*Mus musculus*
Oc	*Oryctolagus cuniculus* (Rabbit)
Os	*Oryza sativa* (Rice)
Rh	*Macaca mulatta* (Rhesus)
Rn	*Rattus norvegicus*
Sp	*Schizosaccharomyces pombe* (Fission Yeast)
Ss	*Sus scroga* (Pig)
Xt	*Xenopus tropicalis* (Frog)

The assay ID suffix indicates the assay placement.

Table 7 Assay ID suffix codes

Suffix	Definition
_m	An assay whose probe spans an exon junction.
_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

Order TaqMan® Gene Expression Assays and TaqMan® Non-coding RNA Assays

TaqMan Gene Expression Assays are available in a variety of formulations. For a comprehensive list of available assays and part numbers, please refer to Table 1 on page 10. All of the assay search methods also provide methods for ordering assays.

If you cannot find a predesigned assay to suit your needs, you can order Custom TaqMan® Gene Expression Assays as described on this page.

Order Custom TaqMan® Expression Assays

Use the Custom TaqMan Assay Design Tool (**www.appliedbiosystems.com/cadt**) to enter and submit sequences for new assay design. The tool also supports submission files created or validated using File Builder software. For details, refer to the *Custom TaqMan® Assays: Design and Ordering Guide* (PN 4367671).

Reorder a Custom TaqMan® Gene Expression Assay

Reorder Custom TaqMan Gene Expression Assays through the Custom TaqMan Assay Design Tool.

1. At **www.appliedbiosystems.com/cadt**, select **Reorder Existing Custom Assays**.
2. In the Search tab, search for existing custom assays by:
   - Assay ID: the ID assigned to a custom assay ordered after Jan 2009. Format: seven alphanumeric characters.
   - Legacy Assay ID: the ID assigned to a custom assay ordered before February 2009. Format: sequence name-target name.
   - Assay Name: the sequence name of any assay designed using the Custom TaqMan Assay Design Tool.
   - Date Range.
   - Sales Order #.

The *Custom TaqMan® Assays: Design and Ordering Guide* (PN 4367671) has details on reordering legacy assays and those designed in the Custom TaqMan® Assay Design Tool, including reordering by e-mail and regular or express mail.

Step 3: Order a candidate endogenous control assay

Search for and order the endogenous control assay using one of the methods described in Step 1 on page 32.

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.

Use Table 8 to order...	Use Table 9 on page 38 to order...
Non-primer limited, FAM™ dye-labeled MGB probe (250 20-µL reactions; PN 4331182)	• Non-primer limited, FAM™ dye-labeled MGB probe (other sizes) • Primer limited, VIC® dye-labeled MGB probe • Primer limited, VIC® dye-labeled, TAMRA™ dye-quenched probe

Table 8 Candidate endogenous control assays (non-primer limited; 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
ABL1	C-abl oncogene 1, receptor tyrosine kinase	Hs00245445_m1	Mm00802029_m1	Rn01436238_g1
ACTB	Actin, Beta, cytoplasmic	Hs99999903_m1	Mm00607939_s1	Rn00667896_m1
B2M	Beta-2-microglobulin	Hs99999907_m1	Mm00437762_m1	Rn00560865_m1
CASC3	Cancer susceptibility candidate 3	Hs00201226_m1	Mm00454629_m1	Rn00595941_m1
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Hs00355782_m1	Mm00432448_m1	Rn00589996_m1
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	Hs00153277_m1	Mm00438168_m1	Rn00582195_m1
EIF2B1	Eukaryotic translation initiation factor 2B, subunit 1 alpha, 26kDa	Hs00426752_m1	Mm00460997_m1	Rn00596951_m1
ELF1	E74-like factor 1 (ETS-domain transcription factor)	Hs00152844_m1	Mm00468217_m1	Rn00585356_m1
GADD45A	Growth arrest and DNA-damage-inducible, alpha	Hs00169255_m1	Mm00432802_m	Rn00577049_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

Gene symbol	Gene name	Human assay ID	Mouse assay ID	Rat assay ID
HPRT1	Hypoxanthine guanine phosphoribosyl transferase 1	Hs99999909_m1	Mm00446968_m1	Rn01527840_m1
IP08	Importin 8	Hs00183533_m1	Mm01255158_m1	Rn00821065_g1
MRPL19	Mitochondrial ribosomal protein L19	Hs00608519_m1	Mm00452754_m1	Rn01425270_m1
MT-ATP6	Mitochondrially encoded ATP synthase 6	Hs02596862_g1	Mm03649417_g1	Rn03296710_s1
PES1	Pescadillo homolog 1, containing BRCT domain (zebrafish)	Hs00362795_g1	Mm00727566_s1	Rn01443731_g1
PGK1	Phosphoglycerate kinase 1	Hs99999906_m1	Mm00435617_m1	Rn00821429_g1
POLR2A	Polymerase (RNA) II (DNA directed) polypeptide A, 220 kDa	Hs00172187_m1	Mm00839493_m1	Rn00574762_m1
POP4	Processing of precursor 4, ribonuclease P/ MRP subunit (S. cerevisiae)	Hs00198357_m1	Mm00481282_m1	Rn02347225_m1
PPIA	Peptidylprolyl isomerase A	Hs99999904_m1	Mm02342430_g1	Rn00690933_m1
PSMC4	Proteasome (prosome, macropain) 26S subunit, ATPase, 4	Hs00197826_m1	Mm00457191_m1	Rn00821605_g1
PUM1	Pumilio homolog 1 (Drosophila)	Hs00206469_m1	Mm00472886_m	Rn00982780_m1
RPL30	Ribosomal protein L30	Hs00265497_m1	Mm01611464_g1	Rn01504620_g1
RPL37A	Ribosomal protein L37a	Hs01102345_m1	Mm01546394_s1	Rn02114291_s1
RPLP0	Ribosomal protein, large, P0	Hs99999902_m1	Mm00782638_s1	Rn01479927_g1
RPS17	Ribosomal protein S17	Hs00734303_g1	Mm01314921_g1	Rn00820807_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 (additional formulations)

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

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	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
	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, 7900HT, and ViiA™ 7 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 Advanced 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).

Table 10 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 Master Mix II, with or no UNG • TaqMan® Universal PCR Master Mix (2×), with or without AmpErase® UNG	TaqMan® Fast Advanced Master Mix
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 RT-PCR

Applied Biosystems offers several chemistries that you can use to perform RT-PCR in one or two steps. See Table 11 on page 41 for a list of kits.

Step 5: Order materials and equipment not included

See "Materials for reverse transcription and PCR" on page 12 for a list of required materials.

Related reagents

Table 11 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 Master Mix II, no UNG	• One 1-mL tube (PN 4440043) • One 5-mL bottle (PN 4440040) • Two 5-mL bottles (PN 4440047) • Five 5-mL bottles (PN 4440048) • Ten 5-mL bottles (PN 4440049) • One 50-mL bottle (PN 4440041)
TaqMan® Universal Master Mix II, with UNG	• One 1-mL tube (PN 4440042) • One 5-mL bottle (PN 4440038) • Two 5-mL bottles (PN 4440044) • Five 5-mL bottles (PN 4440045) • Ten 5-mL bottles (PN 4440046) • One 50-mL bottle (PN 4440039)
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)

Reagent	Description and part number
TaqMan® Fast Advanced Master Mix	• Mini-Pack 1 ×1 mL 100 (PN 4444556) • 1-Pack 1× 5 mL 500 (PN 4444557) • 2-Pack 2 × 5 mL 1,000 (PN 4444963) • 5-Pack 5 × 5 mL 2,500 (PN 4444964) • 10-Pack 10 × 5 mL 5,000 (PN 4444965) • Bulk-Pack 1 × 50 mL 5,000 (PN 4444558)
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 RNA-to-cDNA Kit	50 reactions (PN 4387406)
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 12 Reaction plates and accessories for Applied Biosystems thermal cyclers and real-time PCR systems

Instrument	Reaction plates and accessories
7300 system 7500 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 8-Tube Strips, 0.2-mL, 1000 tubes in strips of eight (PN 4316567) • MicroAmp® Optical 8-Cap Strips, 300 strips (PN 4323032)
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)

Instrument	Reaction plates and accessories
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)
Applied Biosystems ViiA™ 7 system	• 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)

Instrument	Reaction plates and accessories
9700 instrument	• MicroAmp® Optical 96-Well Reaction Plate with Barcode: – 500 plates (PN 4326659) – 20 plates (PN 4306737) • MicroAmp® Optical 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 13 Related gene expression assays and arrays products

Assay or array		For more information...
TaqMan® assays	TaqMan® MicroRNA Assays	**miRNA.appliedbiosystems.com**
	Custom TaqMan® Small RNA Assays	**www.appliedbiosystems.com**
	TaqMan® Pri-miRNA Assays	**mirna.appliedbiosystems.com**
	Custom TaqMan® Probes and Primers‡	**www.appliedbiosystems.com**

Assay or array		For more information...
TaqMan® arrays	TaqMan® Array Cards: • TaqMan® Custom Arrays • TaqMan® Gene Signature Array • TaqMan® Gene Sets	**taqmanarray.appliedbiosystems.com**
	TaqMan® Array Plates§	**www.allgenes.com**
	• TaqMan® MicroRNA Arrays – Human – Rodent • Megaplex™ Primer Pools – Megaplex™ RT Primers – Megaplex™ PreAmp Primers	**miRNA.appliedbiosystems.com**

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

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.

Duplex Reactions

Duplex reactions using TaqMan® Gene Expression Assays

Duplex real-time PCR is the simultaneous amplification and measurement of two target sequences in one reaction. TaqMan® Gene Expression Assays can be used in duplex real-time PCR when using a FAM™ dye-labeled assay in combination with a primer-limited, VIC® dye-labeled assay. However, Applied Biosystems strongly recommends that you validate that your duplex assay combinations provide similar results to your singleplex reactions. When setting a duplex reaction, it is important to:

- Consider the relative expression levels of each target.
- Perform serial dilutions of your sample in both singleplex and duplex reactions, and compare the results for relative expression.
- Select the higher-expressing target as the primer-limited, VIC dye-labeled assay.
- Use TaqMan Gene Expression Master Mix, which has been optimized for duplexing reactions.

For more details on how to validate your duplex assay reactions and interpret the results, refer to the Applied Biosystems Application Note *Factors Influencing Multiplex Reactions* (Publication 136AP04-01 O-081742), available at **www.appliedbiosystems.com**.

Setting up a Duplex Reaction

A duplex reaction is run the same as a singleplex reaction, with the addition of the primer-limited assay:

For each sample (to be run in quadruplicate)	1 reaction (µL)	4 reactions#
TaqMan Gene Expression Assay (20X FAM dye-labeled)	1	5
TaqMan Gene Expression Assay (20X VIC dye-labeled, primer-limited)	1	5
TaqMan Gene Expression Master Mix (2X)‡	10	50
cDNA Template (1-100ng)§	4	20
RNAse-free water	4	20

‡ TaqMan Gene Expression Master Mix has been optimized for duplex reactions. Applied Biosystems does not recommend Universal Master Mix or Fast Master Mix for duplex reactions.

§ Applied Biosystems recommends that the qPCR reaction not be composed of more than 20% of the reverse transcription reaction.

Replicate reactions include 20% excess volume to account for losses during pipetting.

Instructions on how to create and run a plate document/experiment are on page 67 in "Related documentation", which provides a list of resource documents for your instrument.

Background Information

About TaqMan® chemistry

About the probes

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 (Figure 2 through Figure 5) takes place during PCR amplification. This process occurs in every cycle and does not interfere with the exponential accumulation of product.

Figure 1 Legend for Figures 2 through 5

NFQ = Nonfluorescent quencher

MGB = Minor groove binder

R = Reporter

P = Hot-start DNA polymerase

During PCR, the TaqMan MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites (Figure 2).

When the probe is intact (Figure 2 and Figure 3), 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 2 Polymerization

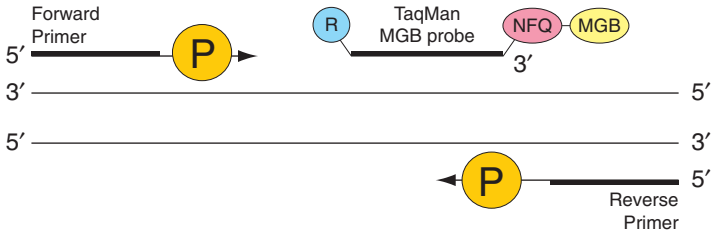
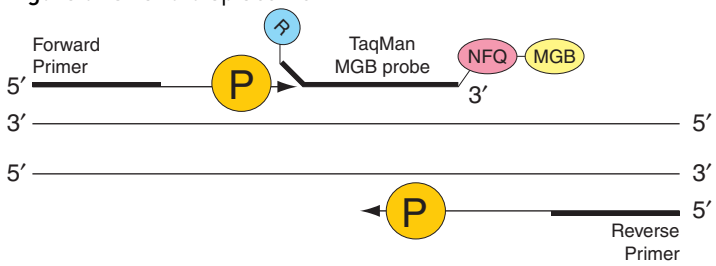
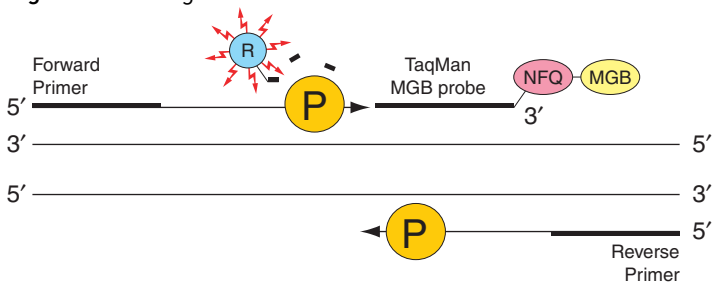


Figure 3 Strand displacement



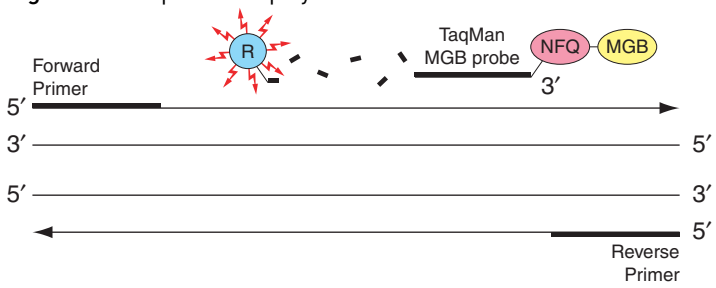
The DNA polymerase cleaves only probes that are hybridized to the target (Figure 4). Cleavage separates the reporter dye from the quencher dye; the separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter. The increase in fluorescence occurs only if the target sequence is complementary to the probe and is amplified during PCR. Because of these requirements, nonspecific amplification is not detected.

Figure 4 Cleavage



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

Figure 5 Completion of polymerization



About the assay information file (AIF)

The assay information file (AIF) contains reference information about your order and technical details of all assays in the shipment. The AIF is included on the Information CD accompanying your order, in a folder labeled with the Rack or Plate ID.

AIF formats

The AIF may be provided in TXT format and/or in both XML and HTML formats, depending on the product line and order date. You can use the:

- HTML-format AIFs as a reference; open them in a Web browser.
- XML- and TXT-format AIFs for electronic data importation and manipulation.

Table 14 Assay information file formats and naming conventions

File format	Filename convention (see Table 16)
HTML	Assay_Info_*ProdLine*_SalesOrder_*XXXX*_RackID_*YYYY*
XML	Assay_Info_*ProdLine*_SalesOrder_*XXXX*_RackID_*YYYY*
TXT	Assay_Info_*ProdLine*_SalesOrder_*XXXX*_RackID_*YYYY* *-or-* *ProdNum_LotNum*_AIF

Table 15 Filename variables

Filename variable	Description
LotNum	The lot number of the assay (more than one lot number may be associated with one manufacturing production number).
ProdLine	The TaqMan assay product line: • TaqMan Gene Expression Assays (TaqMan_GEx) • Custom TaqMan Gene Expression Assays (Custom_TaqMan_GEx) • Custom Plus TaqMan RNA Assays (Custom_TaqMan_ncRNA) • TaqMan Non-coding RNA Assays (TaqMan_ncRNA)
ProdNum	The manufacturing production number.
XXXX...	A unique number assigned to the sales order.
YYYY...	A unique, barcoded number located on various pieces of collateral; the preceding term in the filename may be Rack ID, Plate ID, or Shipping Rack.

AIF field descriptions

Select assay information field descriptions are found in *Understanding Your Shipment* (included on the CD with TaqMan Non-coding RNA Assays and certain TaqMan Gene Expression Assays).

Assay information field descriptions for TaqMan Gene Expression Assays are listed in Table 16, in the order in which they appear in the AIF.

Table 16 Assay information field descriptions for TaqMan® Gene Expression Assays

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	AIT9JN3
Lot Number	A unique identifier assigned to each manufacturing lot	080227T100615	080227T100615
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, Plate ID, or Rack ID	A bar code number on the label of each shipped rack or plate	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	Format: customer-designated sequence name ("MYSEQ")_F	–	MYSEQ_F

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	**Custom TaqMan® Gene Expression Assays**
Forward Primer Sequence	The nucleotide sequence of the forward primer	–	GGACTTGCACGACTAA
Forward Primer Concentration	The concentration of the forward primer (µM)	18	18
Reverse Primer Name	Format: customer-designated sequence name ("MYSEQ")_R	–	MYSEQ_R
Reverse Primer Sequence	The nucleotide sequence of the reverse primer	–	CCGTACGTCAATTGAC
Reverse Primer Concentration	The concentration of the reverse primer (µM)	18	18
Reporter 1 Name	Reporter 1 refers to the oligonucleotide probe. Format: customer-designated sequence name ("MYSEQ")_M.	–	MYSEQ_M
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	–	TTCGAACTGATCAT
Reporter 1 Concentration	The concentration of the reporter 1 probe (µM)	5	5
Reporter 1 Quencher	The quencher used for the reporter 1 probe (for example, Non-fluorescent Quencher [NFQ])	NFQ	NFQ
Reporter 2 Name	Not applicable to TaqMan® Gene Expression Assays	–	–
Reporter 2 Dye			
Reporter 2 Sequence			
Reporter 2 Concentration			
Reporter 2 Quencher			
Context Sequence	The 25-nucleotide sequence surrounding the probe	...NNNNNNNNN...	–
Design Strand	Not applicable to TaqMan® Gene Expression Assays	–	–
Category	The Celera Panther Protein Classification (Level 1) for the gene product	receptor	–
Category ID	A unique, 10-character alphanumeric abbreviation of the Panther category classification	REC1010000	–
Group	The Celera Panther Protein Classification (Level 2) for the gene product	protein kinase receptor	–
Group ID	A unique, 10-character alphanumeric abbreviation of the Panther group classification	1010200000	–
Gene Symbol	The Entrez Gene symbol for the gene	SLC25A14	–

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	**Custom TaqMan® Gene Expression Assays**
Gene Name	The Entrez Gene name for the gene	solute carrier family 25 (mitochondrial carrier, brain), member 14	–
Chromosome	The chromosome containing the gene	9	–
Species	The organism for which the assay was designed	Homo_sapiens	–
Target Exon(s)	The exon(s) that are spanned by the probe	2	–
NCBI Gene Reference	The NCBI transcript identification number(s) corresponding to the assay target	NM_001735	–
NCBI SNP Reference	Not applicable to TaqMan® Gene Expression Assays	–	–
Medline Reference	PubMed references for the gene	–	–
Celera ID *- or -* Assay Name	(*TaqMan Gene Expression Assays*) The unique Celera Discovery System (CDS) assay identification number for the gene	hCT11720402	–
	(*Custom TaqMan Gene Expression Assays*) The customer-designated name of the sequence used for custom assay design (such as "MYSEQ").	–	MYSEQ
Cytogenetic Band	The chromosomal band where the gene is located. If unavailable, then the chromosome number is provided.	9q34	–

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	**Custom TaqMan® Gene Expression Assays**
SNP Type	Not applicable to TaqMan® Gene Expression Assay	–	–
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			

Safety

This appendix covers:

- Chemical safety 60
  - General chemical safety 60
  - SDSs 61
  - Chemical waste safety 61
  - Biological hazard safety 63
- General safety alerts for all chemicals 63

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) 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 Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 61.)
- 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 SDS.
- 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 SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtaining SDSs

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

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS 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 choose

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

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets 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 Safety Data Sheets (SDSs) 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 SDS.
- 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 SDS.
- 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* (**www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**)
- 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

General safety alerts for all chemicals

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

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Documentation and Support

Related documentation

Real-time PCR system	Document	PN/SN
All real-time PCR systems	*Custom TaqMan® Assays: Design and Ordering Guide*	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 Advanced Master Mix Protocol	4444605
	TaqMan® RNA-to-C_T™ 1-Step Kit Protocol	4393463
	White Paper: The Design Process for a New Generation of Quantitative Gene Expression Analysis Tools: TaqMan® Probe-Based Assays for Human, Mouse, and Rat Genes	127WP02-02
	White Paper: Product Stability Study: TaqMan® Gene Expression Assays	127WP03-01
	White Paper: TaqMan® Gene Expression Assays for Validating Hits from Fluorescent Microarrays	127WP01-02
7900HT Fast system Fast or standard sample blocks	*Performing Fast Gene Quantification: Quick Reference Card*	4351892
	Relative Quantitation Using Comparative C_T: Getting Started Guide	4364016
	Performing Fast Gene Quantitation with 384-Well Plates: User Bulletin	4369584
7300, 7500, and 7500 Fast systems	*Relative Quantification: Getting Started Guide*	4347828
ABI PRISM 7700 Sequence Detection System	*Relative Quantitation of Gene Expression*: User Bulletin	4303859
StepOne™ and StepOnePlus™ systems	*Reagent Guide*	4379704
	Relative Standard Curve and Comparative C_T Experiments: Getting Started Guide	4376785

Portable document format (PDF) versions of this and other documents are also available on the *TaqMan Gene Expression Assays* CD.

Note: To open the user documentation included on the TaqMan® Gene Expression Assays CD, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**

Note: For additional documentation, see "Obtaining support".

Obtaining 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, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

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