USER GUIDE

TaqMan®
life technologies™

TaqMan® Array Plates

Fast 96-Well Plates
96-Well Plates
Gene Signature Sets

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life technologies™

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About This Guide

Purpose

The *TaqMan*® Array Plates User Guide: 96-Well Fast Plates, 96-Well Plates, and TaqMan® Gene Signature Sets provides detailed procedures, reference information and troubleshooting techniques for the TaqMan® Array Plates.

User attention words

Two user attention words may appear in this document. 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 or accurate chemistry kit use.

TaqMan® Array Plates

About the plates and assays

TaqMan® Array 96-Well Fast Plates, TaqMan® Array 96-Well Plates, and TaqMan® Array Gene Signature Sets are MicroAmp® Optical 96-Well Reaction Plates, standard or Fast, that contain dried-down TaqMan® Gene Expression Assays. The gene expression assays are a collection of predesigned, gene-specific primer and probe sets for performing quantitative gene expression studies on cDNA. The assays are available for multiple species.

After converting total RNA to cDNA in a reverse transcription (RT) reaction, TaqMan® Array Plates and associated reagents allow you to quantitate RNA expression levels. To do this, you:

- Reconstitute each well of the TaqMan® Array Plate using a mix of standard or Fast master mix and a cDNA sample to a set final volume (10 µL for 96-well Fast plates; 20 µL for 96-well plates).
- Load and run the plates on an Applied Biosystems real-time PCR instrument using either standard or Fast thermal cycling conditions.

Standard versus Fast chemistry

StepOnePlus™, 7500 Fast, and 7900HT Fast Real-Time PCR Systems contain Fast thermal cycling blocks that can perform quantitative PCR using standard or Fast thermal cycling conditions. Applied Biosystems Fast PCR systems use the high-speed thermal cycling blocks, TaqMan® Fast Universal PCR Master Mix (2×), 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 Life Technologies, refer to *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 cycling per run (Standard thermal cycling block)	Fast cycling per run (Fast thermal cycling block)
cDNA quantity	1–100 ng	5–50 ng
Final volume	20 µL	10 µL
TaqMan® Master Mix	• TaqMan® Universal PCR Master Mix • TaqMan® Gene Expression Master Mix (2×)	• TaqMan® Fast Universal PCR Master Mix (2×) • TaqMan® Gene Expression Master Mix (2×)
Plate	• TaqMan® Array Gene Signature Sets: 96-Well Plates • TaqMan® Array 96-Well Plates	• TaqMan® Array Gene Signature Sets: 96-Well Fast Plates • TaqMan® Array 96-Well Fast Plates†

† If necessary, you can run TaqMan® Array 96-Well Fast Plates using universal thermal cycling conditions on Fast thermal cycling blocks using TaqMan® Universal PCR Master Mix or TaqMan® Gene Expression Master Mix (2×).

Available plate types

When you order TaqMan® Array Plates, you choose either predefined or custom plates for standard or Fast cycling:

- Predefined Plates (TaqMan® Array Gene Signature Sets)
  - Contain preplated gene expression assays that detect genes to specific biological pathways, disease states, or have common biological function.
  - Contain at least three candidate control genes and one manufacturing control.
  - Are available in standard or Fast plate formats.
- Custom Plates (TaqMan® Array 96-Well Fast Plates and TaqMan® Array 96-Well Plates)
  - Contain up to 95 inventoried gene expression assays of your choice.
  - Are available in five different plate formats.
  - Are available with or without candidate endogenous control genes (human, mouse, or rat).
  - Contain one manufacturing control.

Candidate endogenous control genes

Custom TaqMan® Array Plates are available with or without the Candidate Endogenous Control Genes. The candidate genes are GAPDH, HPRT1, and GUSB TaqMan® Gene Expression Assays. For more information on selecting endogenous controls, see "About the controls" on page 36.

Available custom plate formats

The TaqMan® Array 96-Well Fast/96-Well Plates Plus Candidate Endogenous Control Genes are available in 96-well plates in the following formats:

- **Format 96** – Contains *one* manufacturing control (18S rRNA) and 95 gene expression assays of your choice.
- **Format 48** – Contains *two sets* of one manufacturing control (18S rRNA) and 47 gene expression assays of your choice.
- **Format 32** – Contains *three sets* of one manufacturing control (18S rRNA) and 31 expression assays of your choice.
- **Format 16** – Contains *six sets* of one manufacturing control (18S rRNA) and 15 gene expression assays of your choice.
- **Format 8** – Contains *twelve sets* of one manufacturing control (18S rRNA) and 7 gene expression assays of your choice.

IMPORTANT! For part numbers and plate layouts, see Table 12 on page 37 and Table 14 on page 40.

About gene expression assays

Each Inventoried TaqMan® Gene Expression Assay (Part no. 4331182) contains sequence-specific, unlabeled primers and FAM™ dye-labeled TaqMan® MGB probe. The assays are reconstituted to a 1X formulation as described in the following table and are designed to run under standard or Fast cycling conditions for two-step RT-PCR.

Plate	Final volume	Compatible master mixes
• TaqMan® Array 96-Well Fast Plates • TaqMan® Array Gene Signature Sets: 96-Well Fast Plates	10 µL	• TaqMan® Fast Universal PCR Master Mix (2X) (for Fast cycling) • TaqMan® Gene Expression Master Mix (2X) (for standard cycling)
• TaqMan® Array 96-Well Plates • TaqMan® Array Gene Signature Sets: 96-Well Plates	20 µL	• TaqMan® Gene Expression Master Mix (2X) • TaqMan® Universal PCR Master Mix

About the information CD

TaqMan® Array Plates are shipped with an Information CD containing files that describe your plate layout and provide details about your assays. It also contains StepOne software, 7500 software, and SDS software setup files (.txt) to help you set up a run on an Applied Biosystems real-time PCR instrument. For more information, see Appendix C on page 29.

Compatible real-time instruments

TaqMan® Array Plates can be used with the following Applied Biosystems real-time PCR systems.

Product	Compatible Applied Biosystems real-time PCR systems
• TaqMan® Array 96-Well Fast Plates • TaqMan® Array Gene Signature Sets: 96-Well Fast Plates	• 7500 Fast System • 7900HT Fast System with 96-well Fast block • StepOnePlus™ System
• TaqMan® Array 96-Well Plates • TaqMan® Array Gene Signature Sets: 96-Well Plates	• 7300 System • 7500 System • 7900HT System with 96-well standard block

Note: TaqMan® Array Fast Plates must be run on real-time PCR systems that contain Fast thermal cycling blocks. Alternately, TaqMan® Array Plates must be run on real-time PCR systems with standard thermal cycling blocks.

Order and store the plates

Order your plates

For details on how to order, refer to the TaqMan® Array Plates product page at:
www.lifetechnologies.com

The general sequence of steps for online ordering is:

1. Choose custom plates and/or predefined plates.
2. Select Fast or standard chemistry.
3. (Custom plates only) Name and describe your experiment, then choose your plate format. Plate formats are described in detail in "Available custom plate formats" on page 35.
4. (Custom plates only) Choose your inventoried assays (Part no. 4331182) for human, mouse, rat, canine, and rhesus genes.

   Inventoried assays are pre-manufactured and available for placement on TaqMan® Array 96-Well Fast/96-Well Plates. For a list of inventoried endogenous control assays, see page 40.

IMPORTANT! If the number of assays that you assign to a TaqMan® Array 96-Well Fast/96-Well Plate is less than the number of wells on the plate, the extra wells will be empty and positioned at the end of the plate.

Note: For information on assay nomenclature, refer to Appendix A, "Assay Nomenclature," on page 25.

Store your plates

TaqMan® Array Plates are shipped at ambient temperature. Store the plates at room temperature (25 °C maximum) or at 2 to 6 °C, protected from light. Keep the plates in the sealed pouch until ready for use.

The Information CD that is shipped with the TaqMan® Array Plates contains .txt files that can help you set up your experiment/plate document. Store the Information CD in a safe place. For more information about the contents of the CD, see page 29.

Barcodes

Each plate has a unique universal barcode that is also printed on its pouch, which allows you to scan the barcode before opening the pouch.

Materials required but not supplied

Master mix

Table 1 lists the TaqMan® master mixes required for use with TaqMan® Array Plates. See Table 3 and Table 4 on page 14 for available master mix quantities.

Table 1 Compatible master mixes

Plate type	Master mixes
TaqMan® Array Gene Signature Sets 96-Well Fast Plates	• TaqMan® Fast Universal PCR Master Mix (2X) (for Fast cycling) • TaqMan® Gene Expression Master Mix (2X) (for Fast or standard cycling)
TaqMan® Array 96-Well Fast Plates	
TaqMan® Array Gene Signature Sets 96-Well Plates	• TaqMan® Gene Expression Master Mix (2X) • TaqMan® Universal PCR Master Mix
TaqMan® Array 96-Well Plates	

Table 2 Volume requirements

Plate type	Master mixes
TaqMan® Array Gene Signature Sets 96-Well Fast Plates	Use 500 µL of master mix per plate (10-µL reactions) or predefined plate (sold individually)
TaqMan® Array 96-Well Fast Plates	
TaqMan® Array Gene Signature Sets 96-Well Plates	Use 1.0 mL of master mix per plate (20-µL reactions) or predefined plate (sold individually)
TaqMan® Array 96-Well Plates	

Master mix part ordering information

Compatible master mixes are available from Life Technologies in the volumes indicated in and Tables 3 and 4.

Table 3 Master mixes for Fast TaqMan® Array Plates

Item	Quantity	Contents	Part number
TaqMan® Fast Universal PCR Master Mix (2X)†	1-Pack	One 5-mL bottle	4352042
	2-Pack	Two 5-mL bottles	4366072
	5-Pack	Five 5-mL bottles	4366073
	10-Pack	Ten 5-mL bottles	4364103
	Bulk Pack	One 50-mL bottle	4367846
TaqMan® Gene Expression Master Mix (2X)	Mini-Pack	One 1-mL tube	4370048
	1-Pack	One 5-mL bottle	4369016
	2-Pack	Two 5-mL bottles	4369514
	5-Pack	Five 5-mL bottles	4369510
	10-Pack	Ten 5-mL bottles	4369542
	Bulk Pack	One 50-mL bottle	4370074

† For use with TaqMan® Assay Fast Plates only.

Table 4 Master mixes for standard TaqMan® Array Plates

Item	Quantity	Contents	Part number
TaqMan® Gene Expression Master Mix (2×)	Mini-Pack	One 1-mL tube	4370048
	1-Pack	One 5-mL bottle	4369016
	2-Pack	Two 5-mL bottles	4369514
	5-Pack	Five 5-mL bottles	4369510
	10-Pack	Ten 5-mL bottles	4369542
	Bulk Pack	One 50-mL bottle	4370074
TaqMan® Universal PCR Master Mix†	1-Pack	One 5-mL bottle	4304437
	2-Pack	Two 5-mL bottles	4364338
	5-Pack	Five 5-mL bottles	4364340
	10-Pack	Ten 5-mL bottles	4305719
	Bulk-Pack	One 50-mL bottle	4326708

† Not for use with TaqMan® Assay Fast Plates.

Plate covers

Table 5 Plate covers

Instrument	Plate cover	Part number
• 7300 System • 7500 System • 7500 Fast System • StepOnePlus™ System	MicroAmp® Optical Adhesive Film: • 25 units • 100 units	 4360954 4311971
7900HT System, 96-Well Standard Block	MicroAmp® Snap-On Optical Film Compression Pad	4333292
	MicroAmp® Optical Adhesive Film: • 25 units • 100 units	 4360954 4311971
7900HT Fast System, 96-Well Fast Block	MicroAmp® Optical Adhesive Film: • 25 units • 100 units	 4360954 4311971

Reverse transcription kit

Table 6 High-capacity reverse transcription kit

Item	Quantity	Part number
High-Capacity cDNA Reverse Transcription Kit	200 reactions	4368814
	200 reactions with RNase Inhibitor	4374966
	1000 reactions	4368813
	1000 reactions with RNase Inhibitor	4374967

Other materials

Table 7 Required consumables and equipment

Item	Part number†
RNase-free, sterile-filtered water	MLS
Centrifuge with plate adapter	MLS
Disposable gloves	MLS
Microcentrifuge	MLS
Pipette tips, aerosol-resistant	MLS
Pipettor(s) of your choice: • Positive-displacement • Air-displacement • Multichannel	MLS
Polypropylene tubes	MLS
Vortexer	MLS

† All materials are available from major laboratory suppliers (MLS).

Life Technologies documents

See "Related documentation" on page 51 for a list of related documents for your real-time PCR instrument.

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:

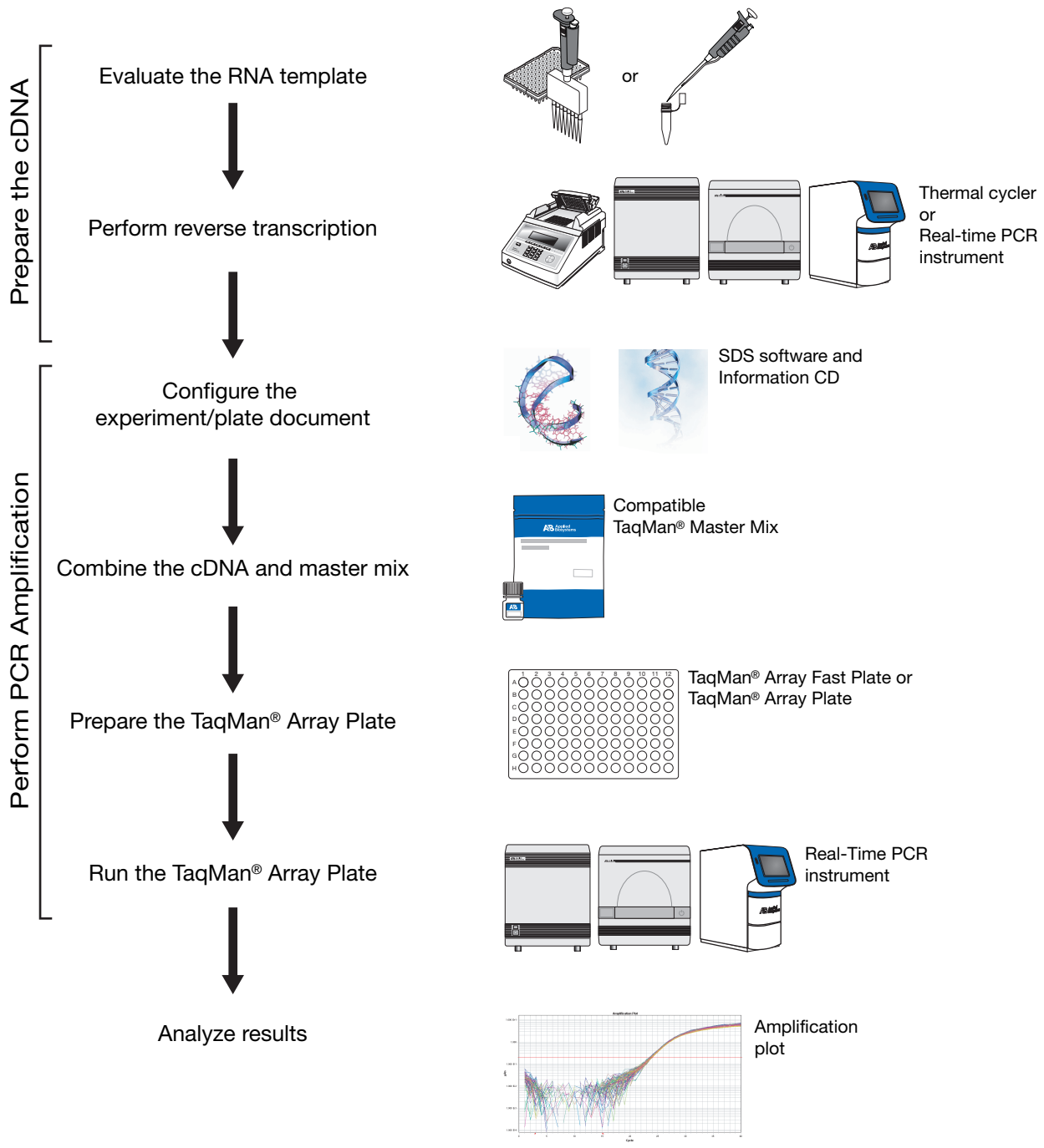
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - 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.

Assay workflow

Figure 1 is a simplified workflow for using TaqMan® Array Plates.

Figure 1 TaqMan® Array Plate workflow



Prepare the cDNA

Evaluate RNA template quality

Before using the TaqMan® Array Gene Signature Set or TaqMan® Array 96-Well Fast/96-Well Plate, you must synthesize single-stranded cDNA from total RNA samples (for a recommended RNA isolation procedure, see "Isolate total RNA" on page 27). We recommend using RNA that is:

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

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

Perform reverse transcription

To obtain cDNA from total RNA samples, we recommend using the High-Capacity cDNA Reverse Transcription Kit. See page 14 for a list of kit part numbers.

The reverse transcription process requires that you:

- Prepare the RT master mix.
- Prepare the reaction plate.
- Perform reverse transcription.

Refer to the *High-Capacity cDNA Reverse Transcription Kit User Guide* (Part no. 4375575) for details. For information on evaluating the cDNA, refer to page 27.

Perform PCR amplification

During PCR amplification, the DNA polymerase from the TaqMan® master mix amplifies target cDNA using sequence-specific primers and TaqMan® MGB probe (6-FAM™ dye-labeled). For information on TaqMan® chemistry, see page 43.

Set up the plate document/ experiment

To set up a plate document (.sds file) or experiment (.eds file):

1. Start the SDS software, 7500 software, or StepOne software.
2. Download the appropriate text (.txt) file from the Information CD to the real-time PCR system computer.

System	Download
7300	*ProdNum*_7300_7500_SDS.txt
7500	• SDS Software – *ProdNum*_7300_7500_SDS.txt • 7500 Software – *ProdNum*_7500_2.0.txt
7500 Fast	• SDS Software – *ProdNum*_7300_7500_SDS.txt • 7500 Software – *ProdNum*_7500_2.0.txt

System	Download
7900HT	*ProdNum*_7900_SDS.txt
7900HT Fast	*ProdNum*_7900_SDS.txt
StepOnePlus	*ProdNum*_StepOne_2.1.txt

3. Refer to the appropriate instrument user guide for information on how to set up the plate document/experiment or create a template from the setup file.

Combine the cDNA and master mix

For the following hazards, see the complete safety alert descriptions in "Chemical safety" on page 46:

1. Mix the TaqMan® master mix thoroughly by swirling the bottle.
2. Thaw your frozen cDNA samples by placing them on ice. After they thaw, resuspend the samples by vortexing, then centrifuge the tubes briefly.
3. For each cDNA sample, label a tube of sufficient size to accommodate the total volume of reaction mix for the number of reactions (see the table on page 19).
4. To each labeled cDNA tube, add the components at the indicated volumes:

Product	Component	Volume per well (µL)					
		1	8†	16†	32†	48†	96†
TaqMan® Array 96-Well Fast Plates or TaqMan® Array Gene Signature Sets: 96-Well Fast Plates (10-µL Reactions)	cDNA + DNase-free water‡	5	45	90	180	270	540
	TaqMan® master mix§	5	45	90	180	270	540
	Total Volume	10	90	180	360	540	1080
TaqMan® Array 96-Well Plates or TaqMan® Array Gene Signature Sets 96-Well Plates (20-µL Reactions)	cDNA + DNase-free water††	10	90	180	360	540	1080
	TaqMan® master mix‡‡	10	90	180	360	540	1080
	Total Volume	20	180	360	720	1080	2160

† Number of reactions; includes 12.5% excess volume.
‡ The final concentration of cDNA is 5 to 50 ng per 10-µL reaction.
§ TaqMan® Fast Universal PCR Master Mix (2X) or TaqMan® Gene Expression Master Mix.
†† The final concentration of cDNA is 1 to 100 ng per 20-µL reaction.
‡‡ TaqMan® Gene Expression Master Mix or TaqMan® Universal PCR Master Mix.

5. Cap the tubes, then gently vortex each tube to thoroughly mix the solution.
6. Centrifuge the tubes briefly to bring the liquid to the bottoms of the tubes.

Prepare the TaqMan® array plate

1. Carefully remove the TaqMan® Array Plate from its packaging.
2. Before removing the plate cover, briefly centrifuge the plate (1000 rpm for 1 min).
3. Remove the cover from the plate, then dispense the appropriate amount of the cDNA and master mix solution to the appropriate wells of the plate.
   - 10 µL if running a 96-well Fast plate
   - 20 µL if running a 96-well plate

   Note: To help remove the cover from the plate, you can use the pick in the CD envelope that is shipped with the plate.

4. Cover the plate using MicroAmp® Optical Adhesive Film.
5. Briefly centrifuge the plate to bring the solution to the bottom of the wells (1000 rpm for 1 min).

IMPORTANT! For optimal results when using TaqMan® Fast Universal PCR Master Mix (2×), prepare the plate on ice and run the plate immediately. If you cannot run the plate within 2 hrs of preparation, refrigerate the reaction plate until you can run it (for up to 24 hrs).

Run the plate on the 7900HT system

Refer to the 7900HT instrument user guide or online help for details on loading and running the plate.

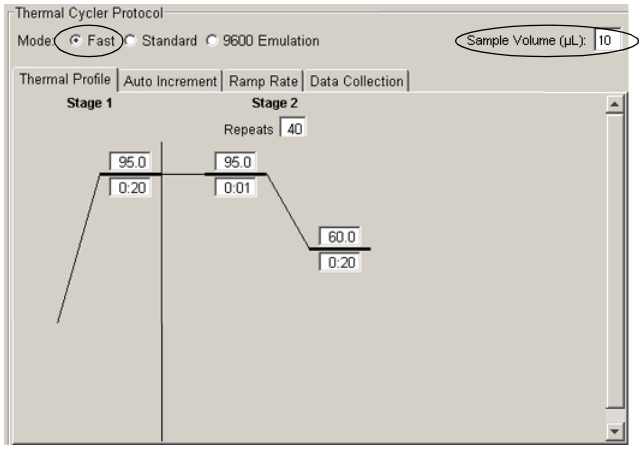
1. If you are running a TaqMan® Array Gene Signature Set 96-Well Plate or TaqMan® Array 96-Well Plate, cover the plate with a MicroAmp Snap-On Optical Film Compression Pad (Part no. 4333292). The pad covers the MicroAmp® Optical Adhesive Film.
2. Load the plate in to the instrument.
3. Specify the thermal cycling conditions for the plate that you are running.

Fast thermal cycling conditions for TaqMan® Array Fast Plates run on a 7900HT Fast system				**Standard thermal cycling conditions for TaqMan® Array Plates run on a 7900HT system**			
Hold†‡	Hold§	PCR (40 cycles)		Hold†	Hold§	PCR (40 cycles)	
		Melt	Anneal/Extend			Melt	Anneal/Extend
50 °C	95 °C	95 °C	60 °C	50 °C	95 °C	95 °C	60 °C
2:00	0:20	0:03	0:30	2:00	10:00	0:15	1:00

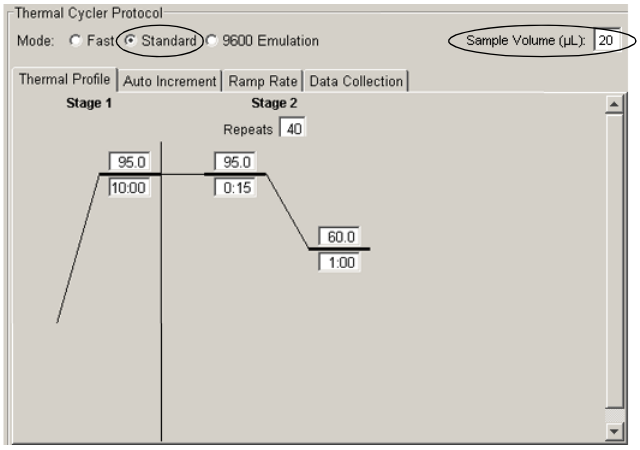
† AmpErase® UNG Activation
‡ Omit the hold if you are using TaqMan® Fast Master Mix, which does not contain AmpErase® UNG.
§ AmpliTaq Gold® Enzyme Activation

Note: TaqMan® Array Fast Plates can be run using Standard run thermal cycling parameters and either TaqMan® Fast Universal PCR Master Mix (2×) or TaqMan® Gene Expression Master Mix.

4. Verify that the correct mode is selected, as shown below.



Fast mode selected



Standard mode selected

5. Set the Sample Volume to:
   - **10** µL if running 96-well Fast plates
   - **20** µL if running 96-well plates
6. Start the run.

Run the plate on the 7300 or 7500 system

Refer to the 7300 and 7500 instrument user guide or online help for details on loading and running the plate.

1. Load the plate in the instrument.
2. Specify the thermal cycling conditions for the plate that you are running.

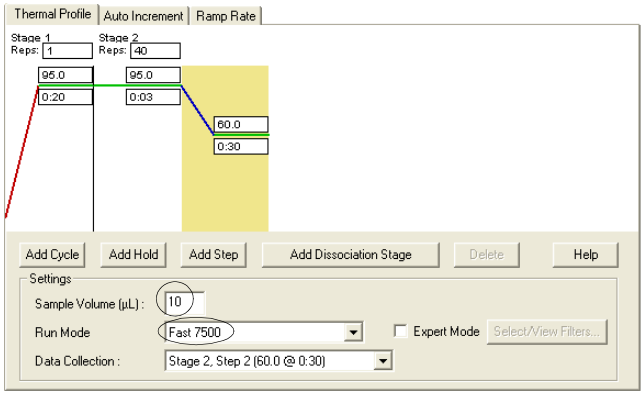
Standard thermal cycling conditions for TaqMan® Array Plates run on a 7300 or 7500 system

Hold†	Hold‡	PCR (40 cycles)	
		Melt	Anneal/Extend
50 °C	95 °C	95 °C	60 °C
2:00	10:00	0:15	1:00

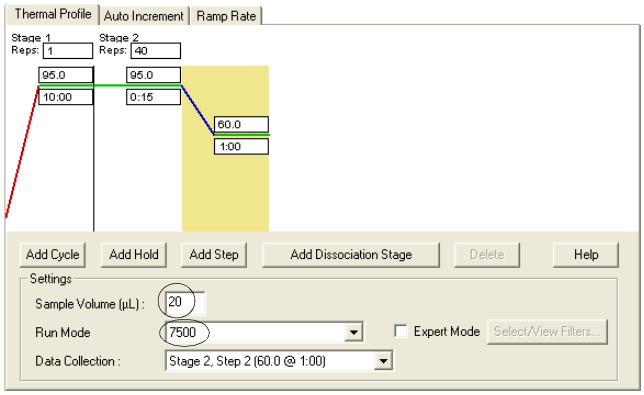
† AmpErase® UNG Activation
‡ AmpliTaq Gold® Enzyme Activation

Note: TaqMan® Array Fast Plates can be run using Standard run thermal cycling parameters and either TaqMan® Fast Universal PCR Master Mix (2X) or TaqMan® Gene Expression Master Mix.

3. Verify that the correct mode is selected, as shown below.



Fast mode selected



Standard mode selected

Note: The figures above show the thermal cycling settings as they appear in the SDS Software v1.4. If you are using the 7500 Software, the settings will appear as shown in "Run the plate on the StepOnePlus™ system or the 7500 Fast system (running 7500 software v2)" on page 22.

4. Set the Sample Volume to **20** µL.
5. Start the run.

Run the plate on the StepOnePlus™ system or the 7500 Fast system (running 7500 software v2)

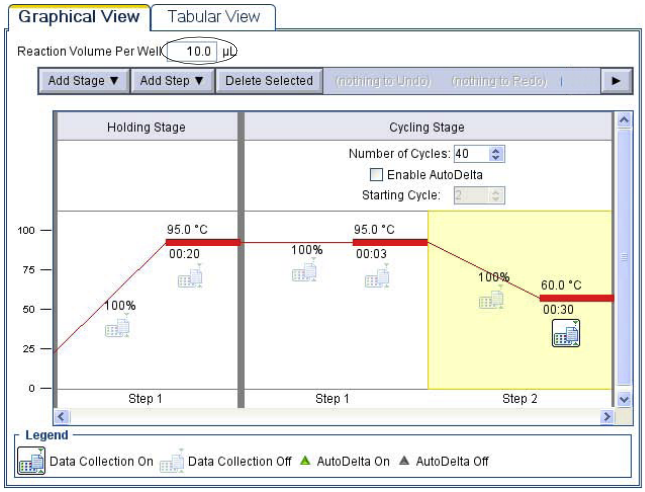
Refer to the StepOnePlus™ instrument user guide or online help for details on loading and running the plate.

1. Load the plate in the instrument.
2. Verify that the correct mode is selected. Select:
   - **Fast** if running 96-well Fast plates
   - **Standard** if running 96-well plates
3. Specify the thermal cycling conditions for the plate that you are running.

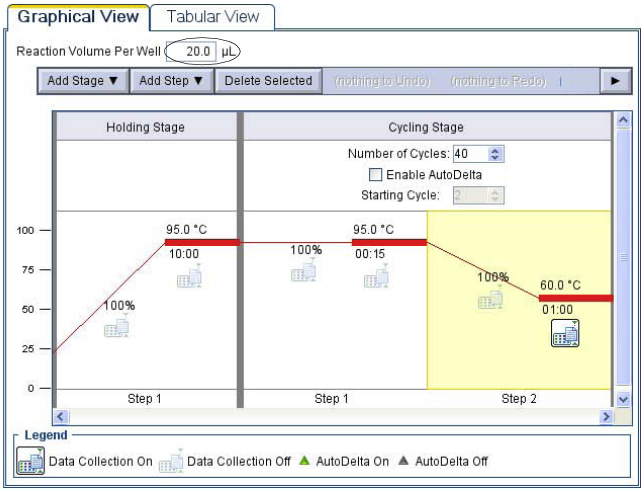
Fast thermal cycling conditions for TaqMan® Array Fast Plates run on a StepOnePlus™ or 7500 systems				**Standard thermal cycling conditions for TaqMan® Array Plates run on a 7500 systems**			
Hold†‡	Hold§	PCR (40 cycles)		Hold†	Hold§	PCR (40 cycles)	
		Melt	Anneal/Extend			Melt	Anneal/Extend
50 °C	95 °C	95 °C	60 °C	50 °C	95 °C	95 °C	60 °C
2:00	0:20	0:03	0:30	2:00	10:00	0:15	1:00

† AmpErase® UNG Activation
‡ Omit the hold if you are using TaqMan® Fast Master Mix, which does not contain AmpErase® UNG.
§ AmpliTaq Gold® Enzyme Activation

Note: TaqMan® Array Fast Plates can be run using Standard run thermal cycling parameters and either TaqMan® Fast Universal PCR Master Mix (2X) or TaqMan® Gene Expression Master Mix.



Fast mode selected



Standard mode selected

4. Set the Sample Volume to:
   - **10** µL if running 96-well Fast plates
   - **20** µL if running 96-well plates
5. Start the run.

Analyze your results

Analyzing the data from 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 analysis depend on the real-time PCR system that you use. Refer to the appropriate instrument user guide for instructions on how to analyze your data.

Resources for data analysis

For more information about analyzing your data, refer to the user documentation for your real-time PCR instrument. See "Related documentation" on page 51 for a list of documents for the compatible instruments.

A

Assay Nomenclature

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	*Arabidopsis thaliana*
Bt	*Bos taurus* (cow)
Ce	*Caenorhabditis elegans*
Cf	*Canis familiaris* (dog)
Dr	*Danio rerio* (zebrafish)
Dm	*Drosophila melanogaster*
Ec	*Equus caballus* (horse)
Gg	*Gallus gallus* (chicken)
Hs	*Homo sapiens*
Rh	*Macaca mulatta* (rhesus)
Mm	*Mus musculus* (mouse)
Oc	*Oryctolagus cuniculus* (rabbit)
Os	*Oryza sativa* (rice)
Rn	*Rattus norvegicus* (rat)
Ss	*Sus scrofa* (pig)

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.

Suffix	Definition
_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).

B

About RNA and cDNA

Isolate total RNA

For optimal performance, we recommend using an Ambion® RNA isolation kit.®

Evaluate the cDNA

Quantity

We recommend that you use:

- 5 to 50 ng of cDNA per 10-µL reaction in the TaqMan® Array Gene Signature Set 96-Well Fast Plates or TaqMan® Array 96-Well Fast Plates.TaqMan® Array Gene Signature Set 96-Well Fast Platesor TaqMan® Array 96-Well Fast Plates.
- 1 to 100 ng of cDNA per 20-µL reaction in the TaqMan® Array Gene Signature Set 96-Well Plates or TaqMan® Array 96-Well PlatesTaqMan® Array Gene Signature Set 96-Well Plates or TaqMan® Array 96-Well Plates.
- The same amount of cDNA for all samples.

DNA quantitation methods

We recommend two DNA quantitation methods:

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

  or

- The TaqMan® RNase P Detection Reagents (Part no. 4316831). You can use your own DNA samples or the TaqMan® DNA Template Reagents (Part no. 401970) to create a standard curve. Refer to *Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR* (Part no. 4371090).

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

C

The Information CD

About the TaqMan® Gene Expression Assays Information CD

When you order TaqMan® Array Plates, 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 array plate. More than one lot number may be associated with one production number.
- *TaqMan®* Array Plates User Guide: 96-Well Fast Plates, 96-Well Plates, and TaqMan® Gene Signature Sets (Part no. 4391016)
- *TaqMan® Array Fast Plates Quick Reference: TaqMan® Array 96-Well Fast Plates and TaqMan® Array Gene Signature Sets 96-Well Fast Plates* (Part no. 4427562)
- *TaqMan® Array Plates Quick Reference: TaqMan® Array 96-Well Plates and TaqMan® Array Gene Signature Sets 96-Well Plates* (Part no. 4391139)

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® Assay Plate 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 8 on page 30 describes the columns of the AIF.

Note: In Table 8, "blank" appears in the Example column for fields that do not apply to TaqMan® Gene Expression Assays or Custom TaqMan® Gene Expression Assays.

Table 8 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 Life Technologies 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 Life Technologies 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
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	20X	20X
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

Field name	Description of content	Examples by product type	
		TaqMan® Gene Expression Assays	Custom TaqMan® Gene Expression Assays
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™ dye	FAM™ dye
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
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 Assays	(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)

Field name		Description of content	Examples by product type	
			TaqMan® Gene Expression Assays	Custom TaqMan® Gene Expression Assays
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)
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			
	African-American			
	Japanese			
	Chinese			
Celera Assembly Build Number				
Location on Celera Assembly				
NCBI Assembly Build Number				
Location on NCBI Assembly				

About the plate layout files

Each Information CD contains two plate layout files, one in HTML format and the other in spreadsheet format. Both files contain identical information.

The plate layout files (*ProdNum*_Platemap.html and *ProdNum*_Platemap.csv) show the position of the assays on the TaqMan® Array Plate. Each plate layout file contains two color-coded maps. The top map shows the gene symbol representing the assay in each well. The bottom map shows the TaqMan Gene Expression Assay ID for each well.

Plate layout files also indicate the:

- Name that you assigned to the plate or the name of the TaqMan® Assay Gene Signature Set.
- TaqMan® Array Plate configuration and part number.
- Lot Number of the plate. Each custom TaqMan® Array Plate is assigned a unique production number. This number appears as part of the file names of the AIF, plate layout, and SDS setup files.

View the plate layout file

Open the .html file to view the layout in a browser or open the .xls file to view the layout in a spreadsheet.

About the setup files

Setup files (*ProdNum*_7900_SDS.txt, *ProdNum*_7500_7300_SDS.txt, *ProdNum*_7500_2.0.txt, or *ProdNum*_StepOne-2.1.txt) contain information specific to your TaqMan® Array Plate, such as the assay IDs and well locations. You can import this file to create SDS plate documents/experiments or templates.

D

Plate Formats

Overview of plates, controls, and formats

Available plates

TaqMan® Array Plates are available as:

- Predefined plates with gene expression assays, multiple endogenous controls, and one manufacturing control, in formats described at: **www.allgenes.com**
- Custom TaqMan® Array Plates and Custom Plus Candidate Endogenous Control Genes (described below).

Available custom plate formats

The Custom TaqMan® Array Plates and Custom Plus Candidate Endogenous Control Genes are available in 96-well plates in the formats in Table 9 below and Table 10 on page 36.

Table 9 Available custom Fast plate formats

Part number	Format	Number of assays			
		User-selected	18s control	Candidate endogenous control genes	Total
Custom TaqMan® Array 96-Well Fast Plate					
4413255	Format 96	95	1	0	96
4413257	Format 48	47	1	0	48
4413259	Format 32	31	1	0	32
4413261	Format 16	15	1	0	16
4413263	Format 8	7	1	0	8
Custom TaqMan® Array 96-Well Fast Plate Plus Candidate Endogenous Control Genes					
4413256	Format 96	92	1	3	96
4413258	Format 48	44	1	3	48
4413260	Format 32	28	1	3	32
4413262	Format 16	12	1	3	16

Table 10 Available custom standard plate formats

Available custom standard plate formats

Part number	Format	Number of assays			
		User-selected	18s control	Candidate endogenous control genes	Total
Custom TaqMan® Array 96-Well Plate					
4391524	Format 96	95	1	0	96
4391526	Format 48	47	1	0	48
4391528	Format 32	31	1	0	32
4413264	Format 16	15	1	0	16
4413266	Format 8	7	1	0	8
Custom TaqMan® Array 96-Well Plate Plus Candidate Endogenous Control Genes					
4391525	Format 96	92	1	3	96
4391527	Format 48	44	1	3	48
4391529	Format 32	28	1	3	32
4413265	Format 16	12	1	3	16

About the controls

Two types of controls are available with the TaqMan® Array 96-Well Fast/96-Well Plates: the manufacturing control and the candidate endogenous control genes. Table 11 shows the symbol and location for each type of control in the TaqMan® Array Plates (shown on page 37).

About the manufacturing control

The manufacturing control on each TaqMan® Array 96-Well Fast/96-Well Plate is 18S ribosomal RNA.

Candidate endogenous control genes

A valid normalization or endogenous control is needed to correct for differences in RNA sampling and sample variation. The ideal control should have a constant RNA level under experimental conditions and be sufficiently abundant across the tissues and cell types being assayed.

Three candidate endogenous control genes are present on the TaqMan® Array 96-Well Fast/96-Well Plate Plus Candidate Endogenous Control Genes: GAPDH, HPRT1, and GUSB. These genes are commonly used as normalization controls because each gene typically produces a constant level of RNA across a wide variety of tissue and cell types.

If you use TaqMan® Array 96-Well Fast/96-Well Plates, or if you want to use additional control genes, see Table 11 for other endogenous control gene assays available from Applied Biosystems. Note that candidate endogenous control genes are not available for all species.

We recommend experimentally validating any candidate gene that you use as an endogenous control.

Table 11 Symbols and locations for controls in TaqMan® Array 96-Well Fast/96-Well Plates

Symbols and locations for controls in TaqMan® Array 96-Well Fast/96-Well Plates

Control symbol	Control name	Gene target	Plates that contain...
MC	Manufacturing Control	18S	• Custom • Custom with Candidate Endogenous Control Genes • Human Endogenous Control
EC1	Candidate Endogenous Control Gene 1	GAPDH	• Custom with Candidate Endogenous Control Genes • Human Endogenous Control
EC2	Candidate Endogenous Control Gene 2	HPRT1	
EC3	Candidate Endogenous Control Gene 3	GUSB	

Note: All TaqMan® Array Gene Signature Sets contain the candidate endogenous controls listed in Table 11.

Custom format plates

Table 12 Formats for custom plates

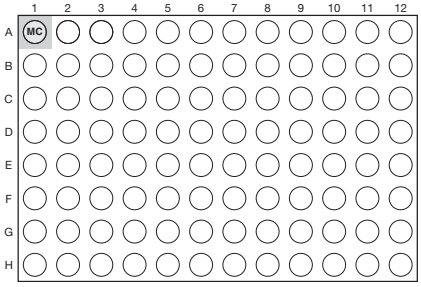
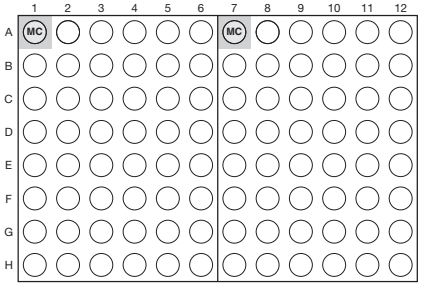
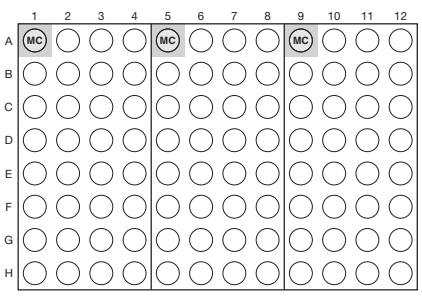
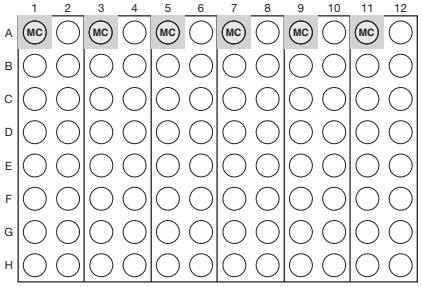
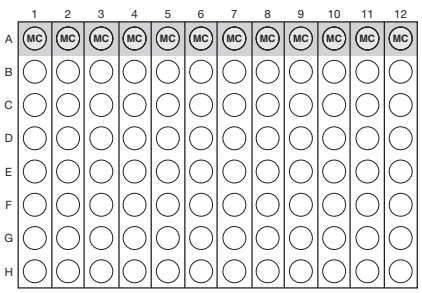
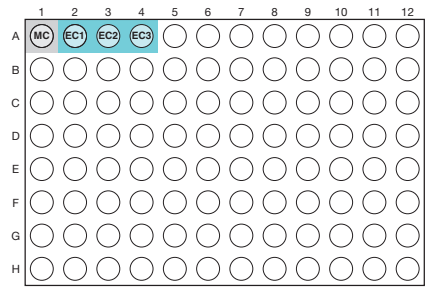
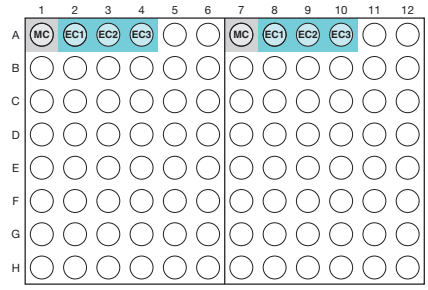
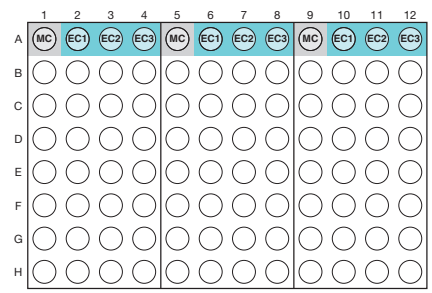
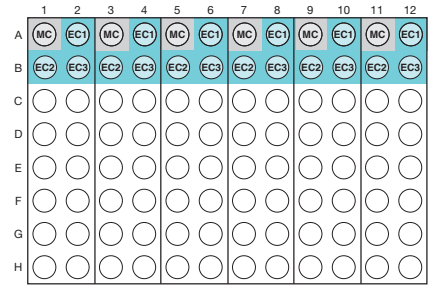
Plate name and part number	Custom plate format	Plate contents
• TaqMan® Array 96-Well Fast Plate, Custom Format 96 (Part no. 4413255) • TaqMan® Array 96-Well Plate, Custom Format 96 (Part no. 4391524)		• Manufacturing control (MC); 18S rRNA • 95 inventoried gene expression assays of your choice
• TaqMan® Array 96-Well Fast Plate, Custom Format 48 (Part no. 4413257) • TaqMan® Array 96-Well Plate, Custom Format 48 (Part no. 4391526)		Two replicates of: • Manufacturing control (MC); 18S rRNA • 47 inventoried gene expression assays of your choice

Plate name and part number	Custom plate format	Plate contents
• TaqMan® Array 96-Well Fast Plate, Custom Format 32 (Part no. 4413259) • TaqMan® Array 96-Well Plate, Custom Format 32 (Part no 4391528)		Three replicates of: • Manufacturing control (MC); 18S rRNA • 31 inventoried gene expression assays of your choice
• TaqMan® Array 96-Well Fast Plate, Custom Format 16 (Part no. 4413261) • TaqMan® Array 96-Well Plate, Custom Format 16 (Part no. 4413264)		Six replicates of: • Manufacturing control (MC); 18S rRNA • 15 inventoried gene expression assays of your choice
• TaqMan® Array 96-Well Fast Plate, Custom Format 8 (Part no. 4413263) • TaqMan® Array 96-Well Plate, Custom Format 8 (Part no. 4413266)		Twelve replicates of: • Manufacturing control (MC); 18S rRNA • 7 inventoried gene expression assays of your choice

Custom format plus endogenous control gene set

Table 13 Formats for the custom plates with Candidate Endogenous Control Genes

Plate name and part number	Custom plate format with candidate endogenous control genes	Plate contents
• TaqMan® Array 96-Well Fast Plate, Custom Format 96 Plus Candidate Endogenous Control Genes (Part no. 4413256) • TaqMan® Array 96-Well Plate, Custom Format 96 Plus Candidate Endogenous Control Genes (Part no. 4391525)		• Manufacturing control (MC); 18S rRNA • Candidate Endogenous Control Genes (EC): – GAPDH (EC1) – HPRT1 (EC2) – GUSB (EC3) • 92 inventoried gene expression assays of your choice
• TaqMan® Array 96-Well Fast Plate, Custom Format 48 Plus Candidate Endogenous Control Genes (Part no. 4413258) • TaqMan® Array 96-Well Plate, Custom Format 48 Plus Candidate Endogenous Control Genes (Part no. 4391527)		Two replicates of: • Manufacturing control (MC); 18S rRNA • Candidate Endogenous Control Genes (EC): – GAPDH (EC1) – HPRT1 (EC2) – GUSB (EC3) • 44 inventoried gene expression assays of your choice
• TaqMan® Array 96-Well Fast Plate, Custom Format 32 Plus Candidate Endogenous Control Genes (Part no. 4413260) • TaqMan® Array 96-Well Plate, Custom Format 32 Plus Candidate Endogenous Control Genes (Part no. 4391529)		Three replicates of: • Manufacturing control (MC); 18S rRNA • Candidate Endogenous Control Genes (EC): – GAPDH (EC1) – HPRT1 (EC2) – GUSB (EC3) • 28 inventoried gene expression assays of your choice
• TaqMan® Array 96-Well Fast Plate, Custom Format 16 Plus Candidate Endogenous Control Genes (Prt no. 4413262) • TaqMan® Array 96-Well Plate, Custom Format 16 Plus Candidate Endogenous Control Genes (Part no. 4413265)		Three replicates of: • Manufacturing control (MC); 18S rRNA • Candidate Endogenous Control Genes (EC): – GAPDH (EC1) – HPRT1 (EC2) – GUSB (EC3) • 12 inventoried gene expression assays of your choice

Endogenous control assays for normalization

To help with normalization, you can choose a human, mouse, or rat endogenous control assay for your custom TaqMan® Array Plate from the list of inventoried endogenous control TaqMan® Gene Expression Assays in Table 14.

Note: Life Technologies recommends experimental validation of any candidate gene to be used as an endogenous control.

Table 14 Inventoried endogenous control assays

Gene name	Human		Mouse		Rat	
	Symbol	Assay ID	Symbol	Assay ID	Symbol	Assay ID
Actin, beta	ACTB	Hs99999903_m1	actb	Mm00607939_s1	Actb	Rn00667869_m1
Beta-2-microglobulin	B2M	Hs99999907_m1	b2m	Mm00437762_m1	B2m	Rn00560865_m1
Cancer susceptibility candidate 3	CASC3	Hs00201226_m1	Casc3	Mm00454629_m1	Casc3	Rn00595941_m1
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	Hs00355782_m1	Cdkn1a	Mm00432448_m1	Cdkn1a	Rn00589996_m1
Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN1B	Hs00153277_m1	Cdkn1b	Mm00438168_m1	Cdkn1b	Rn00582195_m1
E74-like factor 1 (ets domain transcription factor)	ELF1	Hs00152844_m1	Elf1	Mm00468217_m1	Elf1	Rn00585356_m1
Eukaryotic 18S rRNA†	18s	Hs99999901_s1	18S	Hs99999901_s1	18S	Hs99999901_s1
Eukaryotic translation initiation factor 2B, subunit 1 alpha, 26kDa	EIF2B1	Hs00426752_m1	Eif2b1	Mm00460997_m1	Eif2b1	Rn00596951_m1
Glucuronidase, beta‡	GUSB	Hs99999908_m1	gusb	Mm00446953_m1	Gusb	Rn00566655_m1
Glyceraldehyde-3-phosphate dehydrogenase‡	GAPDH	Hs99999905_m1	gapdh	Mm99999915_g1	Gapdh	Rn99999916_s1
Growth arrest and DNA-damage-inducible, alpha	GADD45A	Hs00169255_m1	Gadd45a	Mm00432802_m1	Gadd45a	Rn00577049_m1
Hydroxymethylbilane synthase	HMBS	Hs00609297_m1	hmbs	Mm00660262_g1	Hmbs	Rn00565886_m1
Hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)‡	HPRT1	Hs99999909_m1	hprt1	Mm00446968_m1	Hprt	Rn01527840_m1
Importin 8	IPO8	Hs00183533_m1	ipo8	Mm01255158_m1	Arbp	Rn00821065_g1
Mitchondrially encoded ATP synthase 6	MT-ATP6	Hs02596862_g1	ATP6	Mm03649417_g1	ATP6	Rn03296710_s1
Mitochondrial ribosomal protein L19	MRPL19	Hs00608519_m1	Mrpl19	Mm00452754_m1	Mrpl19	Rn01425270_m1
Peptidylprolyl isomerase A (cyclophilin A)	PPIA	Hs99999904_m1	ppia	Mm02342430_g1	Ppia	Rn00690933_m1
Pescadillo homolog 1, containing BRCT domain (zebra fish)	PES1	Hs00362795_g1	Pes1	Mm00727566_s1	LOC289740	Rn01443731_g1
Phosphoglycerate kinase 1	PGK1	Hs99999906_m1	pgk1	Mm00435617_m1	Pgk1	Rn00821429_g1

Gene name	Human		Mouse		Rat	
	Symbol	Assay ID	Symbol	Assay ID	Symbol	Assay ID
Polymerase (RNA) II (DNA directed) polypeptide A, 220kDa	POLR2A	Hs00172187_m1	polr2a	Mm00839493_m1	Ppib	Rn00574762_m1
Processing of precursor 4, ribonuclease P/MRP subunit (S. cerevisiae)	POP4	Hs00198357_m1	Pop4	Mm00481282_m1	Pop4	Rn02347225_m1
Proteasome (prosome, macropain) 26S subunit, ATPase, 4	PSMC4	Hs00197826_m1	Psmc4	Mm00457191_m1	Psmc4	Rn00821605_g1
Pumilio homolog 1 (Drosophila)	PUM1	Hs00206469_m1	Pum1	Mm00472886_m1	Pum1	Rn00982780_m1
Ribosomal protein L30	RPL30	Hs00265497_m1	Rpl30	Mm01611464_g1	Rpl30	Rn01504620_g1
Ribosomal protein L37a	RPL37A	Hs01102345_m1	Rpl37a	Mm01546394_s1	Rpl37a_ predicted	Rn02114291_s1
Ribosomal protein S17	RPS17	Hs00734303_g1	Rps17	Mm01314921_g1	Rps17	Rn00820807_g1
Ribosomal protein, large, P0	RPLP0	Hs99999902_m1	rplp2	Mm00782638_s1	Rplp2	Rn01479927_g1
TATA box binding protein	TBP	Hs99999910_m1	tbp	Mm00446973_m1	Tbp	Rn01455648_m1
Transferrin receptor (p90, CD71)	TFRC	Hs99999911_m1	tfrc	Mm00441941_m1	Tfrc	Rn01474695_m1
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	YWHAZ	Hs00237047_m1	ywhaz	Mm01158417_g1	Ywhaz	Rn00755072_m1
Ubiquitin C	UBC	Hs00824723_m1	ubc	Mm01201237_m1	Ubc	Rn01789812_g1
V-abl Abelson murine leukemia viral oncogene homolog 1	ABL1	Hs00245445_m1	Abl1	Mm00802029_m1	Abl1	Rn01436238_g1

† This gene is included on all TaqMan® Array Plates.

‡ This gene is included on TaqMan® Array Plate Plus Candidate Endogenous Control Genes.

E

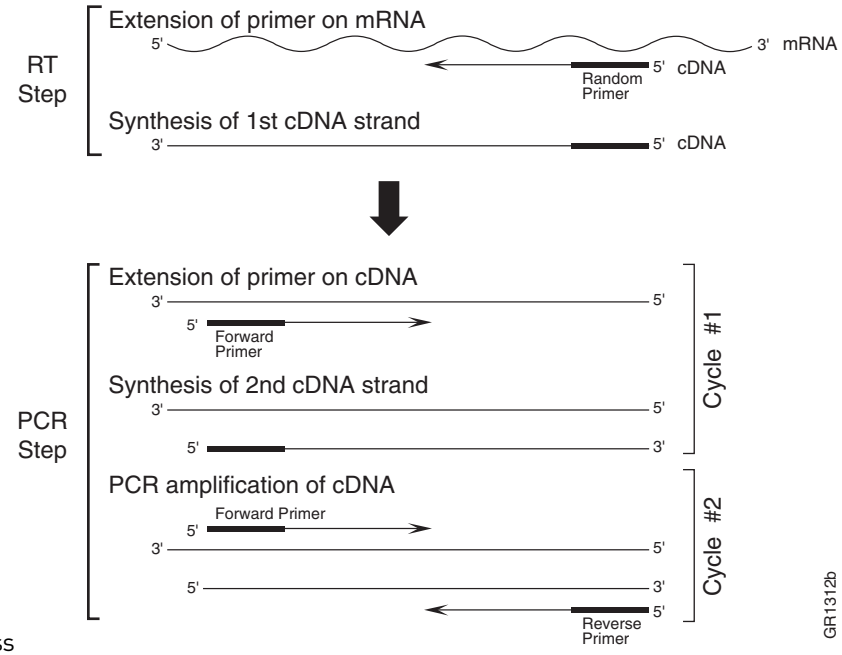
Background Information

TaqMan® chemistry

Two-step RT-PCR

In the reverse transcription (RT) step, cDNA is reverse transcribed from total RNA samples using random primers from the High-Capacity cDNA Reverse Transcription Kit. In the PCR step, PCR products are synthesized from cDNA samples using a PCR master mix. Figure 2 illustrates the two-step RT-PCR assay steps.

Figure 2 Two-step RT-PCR



process

About the probes

The TaqMan® MGB probes contain:

- A reporter dye (6-FAM™ dye) linked to the 5′ end of the probe.
- A minor groove binder (MGB).

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

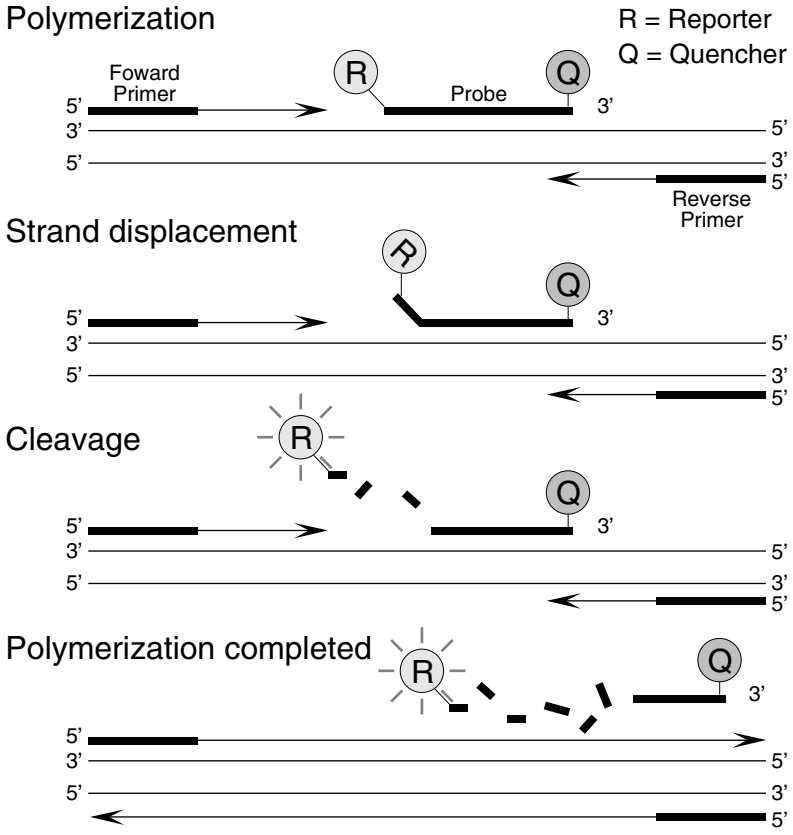
5′ nuclease assay

Figure 3 shows the steps in the 5′ nuclease assay. During PCR:

- Each TaqMan MGB probe anneals specifically to its complementary sequence between the forward and reverse primer sites.
- When the oligonucleotide probe is intact, the proximity of the quencher dye to the reporter dye causes the reporter dye signal to be quenched.

- AmpliTaq Gold® DNA Polymerase or AmpliTaq Gold® DNA Polymerase, UltraPure extends the primers bound to the cDNA template.
- AmpliTaq Gold® enzyme (a 5′ nuclease) cleaves the probes that are hybridized to the target sequence.
- When the hybridized probes are cleaved by AmpliTaq Gold® enzyme, the quencher is separated from the reporter dye, increasing the fluorescence of the reporter dye. Therefore, the fluorescence signal generated by PCR amplification indicates the gene expression level in the sample.

Figure 3 5′–3′ nuclease activity of the DNA polymerase system



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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- 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. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of 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.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the 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

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **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**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Bibliography

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.

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.

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

Documentation and Support

Related documentation

Documents	Part number
All systems	
High-Capacity cDNA Reverse Transcription Kit Protocol	4375575
Real-Time PCR Systems Chemistry Guide	4348358
7300/7500/7500 Fast System	
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantification Getting Started Guide	4347824
Applied Biosystems 7500 Fast Real-Time PCR System Getting Started Guide for $\Delta\Delta C_T$ and Relative Standard Curve Experiments	4387783
Real-Time PCR Systems Chemistry Guide: Applied Biosystems 7900HT Fast Real-Time PCR Systems and 7300/7500/7500 Fast Real-Time PCR Systems	4348358
7500 Software Online Help (within the 7500 Software)	—
SDS Software Online Help (within the SDS Software)	—
7900HTFast System	
Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantification Getting Started Guide	4364016
Life Technologies 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide	4351684
SDS Online Help (within the SDS Software)	—
StepOnePlus™ System	
Applied Biosystems StepOnePlus™ Real-Time PCR System *Getting Started Guide for $\Delta\Delta C_T$ and Relative Standard Curve Experiments*	4376785
StepOnePlus™ Real-Time PCR System Chemistry Guide	4379704
StepOne™ Software Online Help	—

Portable document format (PDF) versions of this guide, the *TaqMan® Array Fast Plates Quick Reference*, and the *TaqMan® Array Plates Quick Reference* are also available on the TaqMan® Array Gene Signature Sets and TaqMan® Array Plates Documentation CD.

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

Note: For additional documentation, see "Obtaining support" on page 52.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

Safety Data Sheets (SDSs) are available from **www.invitrogen.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.invitrogen.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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