

TaqMan[®] Array Micro Fluidic Cards





Introduction

1

2

TaqMan[®] Array Micro Fluidic Cards

Order the TaqMan[®] Array Micro Fluidic Card

Prepare Your

Samples

4

Perform the Experiment

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Contents

	Prefacev
	How to Use This Guidev
	How to Obtain More Information vi
	How to Obtain Support vii
	Important Safety Informationix
	Safety Alert Wordsx
	Chemical Safety Guidelinesx
	About MSDSs xi
	Chemical Waste Safety Guidelines
	Biological Hazard Safety xiii
Chapter 1	Introduction
	Product Overview
	Instrument Overview
	User Guide Overview
Chapter 2	Order the TaqMan [®] Array Micro Fluidic Card
	Order the TaqMan [®] Array Micro Fluidic Card $\dots \dots \dots$
	Available Formats
	Array Information CD
Chapter 3	Prepare Your Samples 31
	Prepare Your Samples
	Store the cDNA Samples
Chapter 4	Perform the Experiment
	Prepare the TaqMan [®] Array Micro Fluidic Card
	Run the TaqMan [®] Array Micro Fluidic Card
	Analyze the Experiment

Appendix A	Troubleshooting
Appendix B	TaqMan [®] Endogenous Control Assays
Appendix C	Required Materials59For Preparing Your Samples60For Preparing the TaqMan® Array Micro Fluidic Card61For Running and Analyzing the TaqMan® Array Micro Fluidic Card62For General Use63
Appendix D	Prevent Contamination65
	Index

Preface

How to Use This Guide

Purpose of This Guide	The <i>Applied Biosystems TaqMan</i> [®] Array Micro Fluidic Cards User Guide provides information for ordering TaqMan [®] Array Micro Fluidic Cards, preparing and running the TaqMan Array cards on an Applied Biosystems 7900HT Fast Real-Time PCR System, then analyzing the results.
Audience	This guide is intended for novice and experienced 7900HT Fast System users who perform quantitation experiments with the TaqMan Array cards using the comparative C_T ($\Delta\Delta C_T$) method.
Text Conventions	This guide uses the following conventions:
	• Bold text indicates user action. For example:
	Type 0 , then press Enter for each of the remaining fields.
	• <i>Italic</i> text indicates new or important words and is also used for emphasis. For example:
	Before running, <i>always</i> prepare fresh master mix.
	• A right arrow symbol () separates successive commands you select from a drop- down or shortcut menu. For example:
	Select File ► Open.
User Attention Words	Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:
	Note: – Provides information that may be of interest or help but is not critical to the use of the product.
	IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.
	Examples of the user attention words appear below:
	Note: There is a fill port on the left arm of each fill reservoir; it is the larger of the two holes.
	IMPORTANT! When performing reverse transcription, do not exceed the amounts of total RNA recommended in the kit protocol.

Safety AlertSafety alert words also appear in user documentation. For more information, see "SafetyWordsAlert Words" on page x.

How to Obtain More Information

RelatedIn addition to this guide, the TaqMan® Array Micro Fluidic Cards User GuideDocumentation(PN 4400263), the documents listed below are available from Applied Biosystems. To
obtain this and additional documentation, see "How to Obtain Support" on page vii.

Title	Part Number
Amplification Efficiency of TaqMan [®] Gene Expression Assays	127AP05
Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide	4351684
Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide	4364016
Applied Biosystems 7900HT Fast Real-Time PCR System Site Preparation Guide	4351923
Applied Biosystems Real-Time PCR Systems Reagent Guide	4387787
High-Capacity cDNA Reverse Transcription Kits Protocol	4375575
High Capacity RNA-to-cDNA Kit Protocol	4387951
High Capacity RNA-to-cDNA Master Mix Protocol	4377474
TaqMan [®] Gene Expression Assays Protocol	4333458
TaqMan [®] Gene Expression Cells-to- C_T^{TM} Kit Protocol	4385117
TaqMan [®] Gene Expression Master Mix Protocol	4371135
TaqMan [®] PreAmp Master Mix Kit Protocol	4384557
TaqMan [®] Universal PCR Master Mix Protocol	4304449
User Bulletin #2: Relative Quantitation of Gene Expression	4303859
Using TaqMan [®] Endogenous Control Assays to Select an Endogenous Control for Experimental Studies (Application Note)	127AP08

Obtaining Information from the Help System

The SDS software Help system describes how to use each feature of the user interface. To access the Help system, you can:

- Click 🕑 in the toolbar
- Select Help > SDS Online Help
- Press F1

To find topics of interest in the Help system, you can:

- Review the table of contents
- Search for a specific topic
- · Search an alphabetized index

Send Us Your
CommentsApplied Biosystems welcomes your comments and suggestions for improving its user
documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" below).

How to Obtain Support

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

At the Support page, you can:

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

Preface How to Obtain Support

Important Safety Information

This section covers:

Safety Alert Words
Chemical Safety Guidelines x
About MSDSs xi
Chemical Waste Safety Guidelines xii
Waste Disposal xii
Biological Hazard Safety xiii

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.

Definitions 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.

Chemical Hazard Warning **WARNING** CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

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

About MSDSs

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

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

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

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

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

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

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

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

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

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

Important Safety Information Biological Hazard Safety

Introduction

This chapter covers:

1

Product Overview	•	2
Instrument Overview		5
User Guide Overview		6

Product Overview

About TaqMan[®] Array Micro Fluidic Cards TaqMan[®] Array Micro Fluidic Cards are 384-well cards preloaded with TaqMan[®] Gene Expression Assays. The TaqMan Array cards allow you to measure gene expression using the comparative $C_T (\Delta \Delta C_T)$ method of relative quantitation. You can run 1 to 8 samples per card, against 12 to 384 TaqMan Gene Expression Assay targets (including controls). Three types of TaqMan Array cards are available from Applied Biosystems:

- TaqMan[®] Array Custom Micro Fluidic Card Customizable card–you choose the assays to include in your card. Select TaqMan Gene Expression Assays from Applied Biosystems collection of Inventoried assays. The TaqMan Array Custom Micro Fluidic Cards are manufactured when ordered.
- **TaqMan® Gene Sets** Customizable card–you choose the assays to include in your card. The TaqMan Gene Sets are a collection of TaqMan Gene Expression Assays that define specific target classes, pathways, or diseases (for example, the TaqMan® Array Human Apoptosis Micro Fluidic Card). Select assays from the TaqMan Gene Sets, then customize the card according to your research needs. TaqMan Array cards with TaqMan Gene Sets are manufactured when ordered.

Note: You can substitute assays within a TaqMan Gene Set with TaqMan Gene Expression Assays from Applied Biosystems collection of Inventoried assays.

• **TaqMan® Gene Signature Array** – Preconfigured card. The TaqMan Gene Signature Arrays contain preselected TaqMan Gene Expression Assays that target specific gene classes (for example, Human Stem Cell Pluripotency markers). The TaqMan Gene Signature Arrays are manufactured and placed in inventory.

A complete list of the TaqMan Gene Expression Assays that are available for each type of TaqMan Array card can be found on the Applied Biosystems web site. For ordering information, see Chapter 2 on page 9.

Key Features

- Small-volume design minimizes sample and reagent consumption.
- Streamlined reaction setup saves time and reduces labor-intensive steps.
- Provides access to high-throughput, 384-well format without liquid-handling robotics.
- Detects two-fold discrimination at the 99.7% confidence level.
- Provides standardization across multiple samples in multiple laboratories.

For More Information

For more information on relative quantitation, refer to *User Bulletin #2: Relative Quantitation of Gene Expression.*



TaqMan Gene Expression Assays are a comprehensive collection of Inventoried and Made to Order Assays (probe and primer sets) for performing quantitation experiments on genes from several species.

Note: Only Inventoried Assays can be placed onto a TaqMan Array card. If you require a Made to Order Assay, please contact your local Applied Biosystems Sales Representative.

The TaqMan Gene Expression Assays:

- Use TaqMan[®] reagents (5' nuclease chemistry) to amplify and detect the target in cDNA samples.
- When possible, can amplify target cDNA without amplifying genomic DNA. The suffix of the assay ID indicates the assay placement (and thus, genomic DNA detection); see "Detection of genomic DNA During Amplification" on page 33.
- Consist of two unlabeled primers and one 6-FAM[™] dye-labeled, TaqMan[®] MGB probe

The TaqMan Gene Expression Assays are:

- Designed with an automated design system.
- Designed and optimized to work with an Applied Biosystems TaqMan[®] master mix using universal thermal cycling conditions.
- Preformulated and ready to use.

Efficiency of TaqMan Gene Expression Assays

A statistically significant number of TaqMan Gene Expression Assays have been tested for PCR efficiency. When tested across a 6-log range, all assays approached 100% efficiency ($\pm 10\%$). Refer to the Application Note *Amplification Efficiency of TaqMan*[®] *Gene Expression Assays* for validation information.

Note: Applied Biosystems does not recommend that you test assay efficiency on the TaqMan Array cards. In order to calculate the efficiency correctly, a dilution series with a minimum of 5 orders of magnitude must be used. With cDNA samples, in most cases it is not possible to obtain results over such a large dynamic range, potentially leading to inaccurate results.

Applications The TaqMan Array cards are recommended for use:

- In relative quantitation experiments using the comparative $C_T (\Delta \Delta C_T)$ method.
- With singleplex PCR. A single primer/probe set is present in each well of the TaqMan Array card. Only one target can be amplified per well.
- In 2-step RT-PCR:
 - In the reverse-transcription (RT) step, you convert total RNA to cDNA. To perform reverse-transcription, Applied Biosystems recommends the High Capacity cDNA Reverse Transcription Kit or the High Capacity RNA-tocDNA Kit.
 - In the PCR step, you combine your cDNA samples and a TaqMan[®] master mix, add this reaction mix to the TaqMan Array card, then run the card on a 7900HT Fast Real-Time PCR System. The instrument must have a TaqMan[®] Array Micro Fluidic Card Thermal Cycling Block installed.

During PCR, the 7900HT Fast System detects the real-time amplification of your targets, then determines relative levels of gene expression from the fluorescence data that are generated during PCR.



The **fill reservoir** includes a **fill port** on the left, and a **vent port** on the right. Use the fill port to add reaction mix to the TaqMan Array card.





Instrument Overview

The TaqMan Array cards must be:

- Centrifuged with a Sorvall[®] or Heraeus centrifuge.
- Sealed with a TaqMan[®] Array Micro Fluidic Card Sealer, then trimmed.
- Run on an Applied Biosystems 7900HT Fast Real-Time PCR System. The instrument must have a TaqMan[®] Array Micro Fluidic Card Thermal Cycling Block installed.

Required Components

Instrument	Component
Sorvall [®] or Heraeus	Sorvall/Heraeus Custom Buckets
centinuge	Card holders
Applied Biosystems 7900HT	Sequence Detection Systems (SDS) Software v2.1 or later
Fast Real-Time PCR System	Note: The SDS software includes a relative quantitation program. Depending on the SDS software version, it may be called RQ Manager or $\Delta\Delta C_T$ Study.
	7900HT TaqMan [®] Array Micro Fluidic Card Upgrade (hardware upgrade kit):
	TaqMan [®] Array Micro Fluidic Card Thermal Cycling Block
	TaqMan [®] Array Micro Fluidic Card Sealer
	 Four centrifuge buckets and card holders (specific to the Sorvall[®] or Heraeus centrifuge)
	 7900HT TaqMan[®] Array Micro Fluidic Card Chemical Installation Kit:
	 Spectral Calibration Kit
	 TaqMan[®] Array Micro Fluidic Card Instrument Verification RNase P Kit
	 Calibration Cards (4 cards)

Before You Begin Before you perform your quantitation experiment, make sure that:

- Within the last 6 months, a background run, pure dye runs, and an instrument performance run (RNase P run) have been performed on the 7900HT Fast System with the TaqMan Array Micro Fluidic Card Thermal Cycling Block installed. For more information, refer to the SDS Online Help or Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide.
- You are familiar with the safety guidelines in the "Safety and EMC Compliance Information" section of the *Applied Biosystems 7900HT Fast Real-Time PCR System Site Preparation Guide*.
- You are familiar with the safety guidelines related to use of your centrifuge, as provided by the centrifuge manufacturer.

User Guide Overview

Workflow



4



Products	For product part numbers and sizes, see Appendix C on page 59.
Documentation	For document titles and part numbers, see "How to Obtain More Information" on page vi.
Services and Support	For the latest services and support information, see "How to Obtain Support" on page vii.



Chapter 1 Introduction User Guide Overview

2

Order the TaqMan[®] Array Micro Fluidic Card

This chapter covers:

Order the TaqMan [®] Array Micro Fluidic Card.	10
Available Formats	22
Array Information CD	26



Order the TaqMan[®] Array Micro Fluidic Card

1. Go to **www.appliedbiosystems.com**.

Order TaqMan[®] Array Custom Micro Fluidic Cards or TaqMan[®] Gene Sets

2. In the I Want to Buy box, click TaqMan[®] Gene Expression Assays, Plates & Arrays.



3. Under Arrays, click 384-Well Arrays (Micro Fluidic Card).

	Gene Expression	Assays & Arrays
	Which gene expression assay TaqMan® Gene Expression As MB).	is right for you? Read our says Product Guide (PDF, 1.0
	Arrays '96-Well Arrays (Microplate) '384-Well Arrays (Micro Fluidic Card) Individual Assays Custom TaqMan® Gene Expression Assays 'TaqMan® Endogenous Controls 'TaqMan® Gene Expression Assays	 TaqMan® Custom Plating Service
4. Click Taq	Man [®] Custom Array o	or TaqMan [®] Gene Sets.



5. (Optional) On the product page, click the **Product Description** tab for more information about the assays and the card layout.



6. Click the Ordering Information tab, then click Configure.



7. On the Store Log In page, enter your user name and password. If you are not a registered user, click **Register Now**, then follow the prompts.

Note: If you are already logged in, the Store Log In page does not appear. Go to the next step.

- 8. Select a format for your TaqMan Array card:
 - **a.** Enter a name and description for your card. You cannot enter more than 24 characters for the card name.
 - b. Select a format. For more information, see "Available Formats" on page 22.
 - c. Click Next.



- **9.** Choose your assays and controls:
 - **a.** Search for assays using one of the following options:
 - Click **Search by Keyword** to display search options. Enter a keyword, select a category, (optional) select additional search criteria (for example, species), then click **Search**. When the search results are returned, select your assays, click **Add to Card**, then click **Return to Configurator**.
 - Click **Search by Batch ID** to display search options. Enter or upload a batch of assay IDs, (optional) select additional search criteria (for example, species), then click **Search**. When the search results are returned, select your assays, click **Add to Card**, then click **Return to Configurator**.

Note: You can place assays from more than one species on the same TaqMan Array card.

• (Recommended for the TaqMan Gene Sets) Click Select a Gene Panel to display a suggested list of gene panels. Select your gene panels, click Add panels to Card, then click Return to Configurator.

Note: For more gene panels, go to: www.appliedbiosystems.com/tools/workflow/.

b. Choose the endogenous controls for your experiment, using any of the search options described in step a above.

Note: Applied Biosystems recommends that you use the TaqMan[®] Endogenous Control Assays to select your endogenous controls. For more information, see Appendix B on page 57 and refer to the Applied Biosystems Application Note Using TaqMan[®] Endogenous Control Assays to Select an Endogenous Control for Experimental Studies.

- **c.** Review the gene symbols added to your TaqMan Array card. If needed, you can:
 - Repeat step a above to add more assays/panels to the card.
 - Select a gene symbol, then click **Remove** to remove assays/panels from the card.
- **d.** In the Manufacturing Controls box, select a gene symbol from the dropdown menu.

Note: The well locations for the controls are predetermined, based on the TaqMan Array card format you selected in step 8 on page 11.

e. Click Next.



- **10.** (Optional) Customize the TaqMan Array card layout:
 - **a.** To change the location of the assays in the card, use the up/down arrows or enter the desired well location.

Note: If you change the well location for one replicate, all other replicates are moved accordingly.

Note: The well locations for the controls are fixed, based on the TaqMan Array card format you selected in step 8 on page 11.



b. Click Update Array Layout.

Note: To return to the previously saved layout, click Reset Layout.

c. Click Next.



- **11.** (Optional) Use one or more of the configuration tools:
 - Click **Save This Design** to save your current TaqMan Array card design to the Applied Biosystems store workspace. If your design is successfully saved, this message appears at the top of the page:

You have successfully added your design to <u>workspace</u>.

Note: For more information on the Applied Biosystems store workspace, click the **workspace** link shown in the message.

- Click **Export Assay List**, then follow the prompts to export a list of your current assays to a simple text application (*.txt file).
- Click **Print Array Layout**, then select **File Print** to print your current TaqMan Array card layout.





- **12.** Review and order your TaqMan Array card:
 - a. Review the card details. If needed, click Previous to change the information.
 - **b.** Enter the quantity of cards to order. The minimum quantity you can order is 10 cards.

Note: After the minimum quantity has been fulfilled, you can order TaqMan Array cards in increments of one.

c. Select your basket from the dropdown menu.

	🗸 🕘 Review & Orde	r	
			C Previous
	Array Details		
	Name	Test	
	Description		
	Format	Format 96a	
12a —	Species Selected	Homo sapiens	
	Assigned Wells	95	
	Unique Genes	94	
	Empty Wells	0	
	Control Assays	18s rRNA	
12b ——	Select Package Quantity *	(Minimum quantity of 10)	
	* Required Field		
12c —	My Basket06/20/2008	Add to Basket	
		12d	S Previous
	Trademark		



- d. Click Add to Basket. You can:
 - Repeat step 8 on page 11 through step 12 on page 15 to continue ordering TaqMan Array cards.
 - Click Shopping Basket to check out.



Applied Biosystems ships the TaqMan Array cards at ambient temperature. Upon receipt, store the cards at 2 to 8 °C. The cards are stable for up to 2 years from the manufacturing date.

Order TaqMan[®] Gene Signature Arrays

- 1. Go to www.appliedbiosystems.com.
- 2. In the I Want to Buy box, click TaqMan[®] Gene Expression Assays, Plates & Arrays.



3. Under Arrays, click 384-Well Arrays (Micro Fluidic Card).

Gene Expression Assays & Arrays

Which gene expression assay is right for you? Read our TaqMan® Gene Expression Assays Product Guide (PDF, 1.0 MB). • Arrays • Ge-Well Arrays (Microplate) • TaqMan® Custom • Plating Service

- · '384-Well Arrays (Micro Fluidic Card)
 Individual Assays
- Custom TaqMan® Gene Expression Assays
- TaqMan® Endogenous Controls
- 'TaqMan® Gene Expression Assays
- 4. Click TaqMan[®] Gene Signature Arrays.



5. On the TaqMan[®] Gene Signature Arrays page, select the appropriate Gene Signature Array.



- **6.** (Optional) On the product page, click the **Product Description** tab for more information about the assays and the card layout.
- 7. Click the Ordering Information tab.
- **8.** Log in:
 - a. Click Log In.

Note: If you are already logged in, the Log In prompt does not appear. Go to the next step.

b. On the Store Log In page, enter your user name and password. If you are not a registered user, click **Register Now**, then follow the prompts.

7	Ordering Information	Product Description		ature/Support
		The TaqMan(contains assa addition to 1 well array.	8 Human ABC Tra ays for 50 human 4 endogenous cor	nsporter Array genes in ntrols in a 384-
8a	Please Log In to add configure a produ your country.	l products to y ct, or to view j	our Shopping Bas products available	sket/Favorites, for purchase in
	Product Nam	e	Part Number	Quantity/ Package
	TaqMan® Hum Transporter Arr	an ABC ay	4378700	4 cards
	Note: See user's m license, and tradem for use in diagnostic Trademark	anual or packa ark informatio :s procedures.	ige insert for limit n. For Research U	ed label se Only. Not

- 9. Order your TaqMan Array card:
 - **a.** Select the card.
 - **b.** Select your basket from the dropdown menu.



	Ordering Information	Product Description	Literature	/Support			
		The TaqMan® Hum contains assays for addition to 14 endo well array.	an ABC Transp 50 human gen genous control	orter Array ies in Is in a 384-			
	You can view your c Basket or Favorites.	ontract pricing for pr	oducts in your	Shopping			
	My Basket11/2 💙	Add to Basket	My Favor 💌				
		Add to Favorites					
	Product Name	e Part Number	Quantity/ Package	List [®] Price [View Your Price]			
9a	✓ TaqMan® Human ABC Transporter Array	4378700	4 cards				
9b	My Basket11/2 ¥	Add to Basket	My Favor 🛩	rites			
	9c						
	Note: See user's manual or package insert for limited label license, and trademark information. For Research Use Only. Not for use in diagnostics procedures.						
	Trademark						

- c. Click Add to Basket. You can:
 - Repeat step 3 on page 16 through step 9 on page 17 to continue ordering TaqMan Gene Signature Arrays.
 - Click Shopping Basket to check out.



Applied Biosystems ships the TaqMan Array cards at ambient temperature. Upon receipt, store the cards at 2 to 8 °C. The cards are stable for up to 2 years from the manufacturing date.



Reorder TaqMan[®] Array Custom Micro Fluidic Cards or TaqMan[®] Gene Sets

There are two ways to reorder the same TaqMan[®] Array Custom Micro Fluidic Cards or TaqMan[®] Gene Sets:

 Import a saved design from within the Configurator (this page) – To use this method, you must have previously saved a design (step 11 on page 14) or added an order to your shopping basket (step 12 on page 15).

Note: You can use this method if you have not yet submitted the order (that is, you added the order to your shopping basket, but did not check out).

• Reorder from your shopping basket (page 21) – To use this method, you must have previously submitted an order (step 12 on page 15).

Import a saved design

- 1. Go to www.appliedbiosystems.com.
- 2. In the I Want to Buy box, click TaqMan[®] Gene Expression Assays, Plates & Arrays.



3. Under Arrays, click 384-Well Arrays (Micro Fluidic Card).



4. Click TaqMan[®] Custom Array or TaqMan[®] Gene Sets.





5. On the product page, click the Ordering Information tab, then click Configure.

1

TaqMan® Gene Sets are genes grouped by pathway or disease target class which can l ordered separately, as a custom plate, or configured directly onto TaqMan® Arrays that enable you to perform hundreds of rea time PCR reactions simultaneously on the Applied Biosystems 7900HT Fast Real-Time PCR System. Simple to implement, TaqMan® Gene Sets are customizable, and when on a TaqMan Array require minimal amounts of sample, allow for 1 to 8 sample to be run in parallel against multiple TaqMan® Gene Expression Assay targets, and don't require liquid-handling robotics o complex pipetting.
How to Order Open the Ordering Guide (4.8 MB) and follo the steps to add TaqMan® Gene Sets to you

6. On the Store Log In page, enter your user name and password.

Note: If you are already logged in, the Store Log In page does not appear. Go to the next step.

7. In step 1 of the Configurator, click Import Saved Design.

	Product Description	Literature/Support	Related Products	Configure
Configuration Tools	∎ Import Saved Design	Compatible Instruments		
Select a	Format			
format, click View A	, Arrav Lavout. To se	e a layout for all array fo	ormats in one document	
the All_Array_Maps	cribe Your Ta	aqMan Array		, piease downio
the All_Array_Maps Name & Des Name *	s.pdf(57kb). cribe Your Ta	aqMan Array		, piease downio
the All_Array_Maps Name & Des Name * Description	.pdḟ(57Kb). cribe Your Ta	aqMan Array		, piease downio

8. On the Designs page, click the name of the design you want to import. You are returned to step 4 of the Configurator (Review & Order).



9. In the Configurator, review and order the TaqMan Array card according to step 12 on page 15, or click **Previous** to make changes.

Reorder from your shopping basket

- 1. Go to www.appliedbiosystems.com.
- 2. At the top of the Home page, from the My Baskets/Orders dropdown menu, select **Order History/Reorder**.

Applied Biosystems			- Charles Cat	ß		Mr. D Mathematica		
Sinted states		 store Log Out 	🚱 My Account 🔸 🚆		View My Basket	Calar o	rder	
Home	Products	Applications & Technologies	Services	Support	Learnii	Manage Shopping Baske	ts re Help	
					Order History/Reorder	dm		
Welcome L	Welcome Laurie Swenson. [Not Laurie Swenson?]			Enter search term Favorit		Favorites		2
						Promotions/Quotes		
<u> </u>	1.00					Workspace		

3. On the Store Log In page, enter your user name and password.

Note: If you are already logged in, the Store Log In page does not appear. Go to the next step.

4. Select the TaqMan Array card to reorder, then click **Reorder** at the top-right of the page.


Available Formats

The table below provides a list of available TaqMan[®] Array Micro Fluidic Card formats for selected TaqMan[®] Gene Expression Assays. Illustrations of each format follow.

Format	Part No.	No. of Target Assays	Minimum Order No.‡	Mandatory Manufacturing Controls	Maximum No. of Samples per Card	Minimum No. of Ports per Sample (8 per card)
12	4342247	11	10	1	8 (quadruplicates)	1
16	4346798	15	10	1 8 (triplicates)		1
24	4342249	23	10	1 8 (duplicates)		1
32	4346799	31	10	1 4 (triplicates)		2
48	4342253	47	10	1	8 (no replicates)	1
64	4346800	63	10	1	2 (triplicates)	4
96a	4342259	95	10	1	4 (no replicates)	2
96b	4342261	95	10	1	2 (duplicates)	4
192	4346802	191	10	1	1 (duplicates)	8
384	4342265	380	10	1 (4 replicates)	1 (no replicates)	8

‡ After the minimum quantity has been fulfilled, you can order TaqMan Array cards in increments of one.

Format 12 11 target assays + 1 mandatory manufacturing control

8 unique samples



Format 16 15 target assays + 1 mandatory manufacturing control

8 unique samples



Format 24 23 target assays + 1 mandatory manufacturing control 8 unique samples

unique samples



Format 32 31 target assays + 1 mandatory manufacturing control

Replicates Port 1 — 1 2 3 2 4 5 3 6 7 4 8 12 14 15 16 18 19 20 21 22 23 24 9 10 11

4 unique samples



Format 48 47 target assays + 1 mandatory manufacturing control

8 unique samples



Format 6463 target assays + 1 mandatory manufacturing control

2 unique samples



Format 96a 95 target assays + 1 mandatory manufacturing control

4 unique samples



Format 96b 95 target assays + 1 mandatory manufacturing control

2 unique samples



Format 192 191 target assays + 1 mandatory manufacturing control 1 unique sample



Format 384 380 target assays + 4 mandatory manufacturing controls

1 unique sample





Array Information CD

The Array Information CD is shipped with your TaqMan Array card. It includes the following files:

- SDS Setup File (this page)
- Array Map File (page 27)
- Assay Information File (AIF) (page 27)
- *readme* text file
- PDF file of this guide, the TaqMan [®]Array Micro Fluidic Cards User Guide
- PDF file of the *Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide*

SDS Setup File

The SDS Setup File is a text file (*.txt) included on the Array Information CD. The SDS Setup File contains information specific to your TaqMan Array card, such as detector/reporter and task information for each well.

When you set up the SDS plate document for your TaqMan Array card in the SDS software, Applied Biosystems recommends that you import the SDS Setup File (see step 4 under "Set Up the SDS Plate Document" on page 46). The SDS software uses the information in the SDS Setup File to configure the plate grid and setup table in the SDS plate document. The figure below shows an SDS plate document after an SDS Setup File has been imported.

IMPORTANT! Modifying the contents of the SDS Setup File can corrupt the file, making the file unusable (that is, you will not be able to access information for the TaqMan Array card).

The SDS software displays well information if you leave the cursor over the well.

1 3	2 3 4 5 6	7 8 9 10 11 12 1	3 14 15 1	6 17 18	3 19 20) 21 22	23 24	1	Setup	Instrument	d .					
A									10/-11/-1							
8									vven(s)	- N				_		
5	ample Name: Sa	ample01							Sample	e Name: S	ample01					
Ď	etectors:	20 mr 1 Tanant					66	-	Use		Detector		Reporter		Task	Color
	CTD-H59999999	JS_mi - Target								18S-	Hs99999901 s1		EAM			
								-	X	ACTE	Hs99999903 m1		EAM	Target		
								_	Г	ACTC	Hs00606316 m1		FAM			
3									Г	AFP-I	Hs00173490 m1		FAM			
									Г	BRIX-	Hs00217848 m1		FAM			
									Г	CD34-	Hs00156373 m1		FAM			
									Г	CD9-	Hs00233521_m1		EAM			
									Г	CDH5	Hs00174344_m1		EAM			
									Г	CDX2-	Hs00230919_m1		EAM			
									Г	CGB-	Hs00361224_gH		FAM			
									Г	COL1A	1-Hs00164004_m1		FAM			
									Г	COL2A	1-Hs00156568_m1		FAM			
									Г	COMME	3-Hs00201350_m1		FAM			
									Г	CRABP	2-Hs00275636_m1		FAM			
									Г	CTNNB	1-Hs00170025_m1		FAM			
_								-	Г	DDX4	Hs00251859_m1		FAM			
									Г	DES-I	Hs00157258_m1		FAM			
4								P	Г	DNMT3	B-Hs00171876_m1		FAM			
									Г	EBAF	-Hs00745761_s1		FAM			
	tinge: None	× 6							Г	EEF1A	1-Hs00742749_s1		FAM			
010 000	ango, prono	_ ~							Г	EOMES	S-Hs00172872_m1		FAM			
osit	Elag Sample	Detector	Task	EOS	HMD	LME	EW	BE 2		FGF4	Hs00173564_m1		FAM			
	Comple01	ACTP 4/00000002 mt	Toract					<u> </u>	F	FGF5-	Hs00170454_m1		FAM			
2	Sample01	ACTC-HeDDE06316_m1	Target						1	FLI1-	HsUU1/66/3_m1		FAM			
5	Sample01	AER H=00173490 m1	Target							EN1-	HsUU2//5U9_m1		FAM			
	Sample01	BRIX-He00217848 m1	Tarnet	-						FOXA2	-HsuU232/64_m1	-	FAM			
	Sample01	CD34.He00156373_m1	Tarnet	-				- 000	1	FOXD:	5-HSUU255287_s1	_	FAM			
	Sample01	CD9-He00233521_m1	Tarnet	-				- 22	1	GABRB	3-HsUU241459_m1		FAM			
	Sample01	CDH5.He00174344_m1	Target	-				- 333		GAL-	HsUU544355_m1		FAM			
3	Sample01	CDV2.He00230919 m1	Target	-						GAPD	Hs99999905_m1		FAM			
4	Sample01	CGR_Hs00361224_gH	Tarnet	-						GATA	HSUU1/14U3_m1	-	FAM			
10	Sample01	COL1A1-He00164004 m1	Tarnet	-				- 222	1	GATAB	HSUU232018_m1	_	FAM			
11	Sample01	185,He99999901 e1	Endogeno	-				- 000	1	GBX2-	HSUU230965_m1		FAM			
2	Sample01	COL2A1.He00156568 m1	Tarnet	-				- 33	1	GCG-	HsUU174967_m1		FAM			
3	Sample01	COMMD3.He00201350_m1	Target	-				-		GCM1	-HsUU172692_m1		FAM			
14	Sample01	CRARP2-He00275636_m1	Tarnet	-				- 19		GDF3	HSUU220998_m1	-	FAM			
15	Sample01	CTNNE1-He00170025 m1	Tarnet	-				- 333	-	GFAP	-HSUU15/6/4_m1	-	FAM			
16	Sample01	DDY4.He00251859_m1	Tarnet	-				- 000		GRB/	-Hallen7999 61		FAM			
17	Sample01	DES-Hs00157258 m1	Target	-					Add	Detector	Clear Co					
10	SampleO1	DNIMT2R H=00171976 m1	Torgot	-	-											
18	1.5.41111111111111111111111111111111111															
9	Sample01	EBAE-Hc00745761 c1	Target					-	Passiv	e Reference	ROX 🔻					



Array Map File

Contents An Array Map File contains two color-coded maps that show the position of the assays on the TaqMan Array card. The top map shows the replicate distribution of assay gene symbols for each well. The bottom map shows the TaqMan Gene Expression Assay ID numbers.

Array Map Files also indicate the:

- TaqMan Array card configuration and part number.
- Production number of the TaqMan Array card. Each TaqMan Array card is assigned a unique production number. This number appears as part the AIF, Array Map File, and SDS Setup File names.
- **Formats** Each Array Information CD contains two Array Map Files, one in HTML format, and the other in spreadsheet format.

То	open	using			
View a map	<prodnum>[‡]_cardmap.html</prodnum>	your web browser.			
Print a map	<prodnum>[‡]_cardmap.xls</prodnum>	Microsoft [®] Excel [®] software.			

‡ Where *<prodNum>* is the production number of the TaqMan Array card.

Assay Information File (AIF)

About the AIF The Assay Information File (AIF) is a text file that describes the TaqMan Array card: AIF_*ProdNum*.txt where *ProdNum* is the manufacturing production number

To view the AIF as a spreadsheet in Microsoft[®] Excel[®] Software:

- **1.** Load the Array Information CD into the CD drive.
- 2. Navigate to the drive that contains the Array Information CD.
- 3. Click, then right-click AIF_ProdNum.txt, then select Open with Excel.



AIF columns The table below describes the columns of the AIF.

Note: In the table below, "N/A" appears in the Example column for fields that do not apply to TaqMan Array cards.

Field name	Description of content	Example			
Customer Name	Your organization or institution	University XYZ			
(Sales) Order Number	A unique number that identifies the Applied Biosystems sales order	1234567890			
Ship Date	hip Date The date when the assay was packaged for shipment				
Delivery Number (Shipment ID)	A unique bar code number that identifies the shipment Note: The shipment ID also appears in the plate ID.	N/A			
Part Number	A number that identifies the product line	4331182			
Product Type	The Applied Biosystems product line associated with the assay	TaqMan [®] Gene Expression Assays			
Assay ID	An alphanumeric string that identifies the assay	Hs00154374_m1			
Lot Number	A unique alphanumeric string that identifies the manufacturing batch to which the assay belongs	A3579			
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)	384-well, 8-port Micro- Fluidic Card			
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.	N/A			
Vial/Tube Type	The type of vial or tube that contains the assay	N/A			
Vial/Tube ID	A unique, 10-digit bar code number on the bottom of each assay vial or tube that identifies it	N/A			
Well Location on the Shipping Rack or Plate	The location of the assay on the associated shipping rack or plate	A1			
Assay Mix Concentration	The concentration of the assay, including both primers and probe	2X			
Forward Primer Name	The name of the forward primer, assigned by the design software, that consists of the assay ID plus an "F" suffix	N/A			
Forward Primer Sequence	The nucleotide sequence of the forward primer	N/A			
Forward Primer Concentration	The concentration of the forward primer (µM)	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	N/A			
Reverse Primer Sequence	The nucleotide sequence of the reverse primer	N/A			
Reverse Primer Concentration	The concentration of the reverse primer (µM)	18			



Field name	Description of content	Example
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:	N/A
	 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.	
Reporter 1 Dye	The reporter dye label for the reporter 1 probe	FAM™
Reporter 1 Sequence	The nucleotide sequence of the reporter 1 probe	N/A
Reporter 1 Concentration	The concentration of the reporter 1 probe (μM)	5
Reporter 1 Quencher	The quencher used for reporter 1 probe (for example, Minor Groove Binder-Non Fluorescing Quencher [MGB-NFQ])	NFQ
Reporter 2 Name	Not applicable to TaqMan [®] Gene Expression Assay.	N/A
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)	AGGAAACGTCTGGGCGAT GACAACC
Design Strand	Indicates the strand used to design the probe:	N/A
	 Forward – The probe binds to the same strand as the forward primer. 	
	 Reverse – The probe binds to the same strand as the reverse primer. 	
Category	The Celera Panther Protein Classification (Level 1) for the gene	Nucleic acid binding
Category ID	A unique, 10-character alphanumeric abbreviation of the Panther category classification for the assay	NUC 1090000
Group	The Celera Panther Protein Classification (Level 2) for the gene	Replication origin binding protein
Group ID	A unique, 10-character alphanumeric abbreviation of the Panther group classification for the assay	1090300000
Gene Symbol	The Entrez Gene symbol for the gene	CDC6
Gene Name	The Entrez Gene name for the gene	Cell division cycle 6 homolog (S. cerevisiae)
Chromosome	The chromosome containing the gene	17
Species	The organism for which the assay was designed	Homo_sapiens
Target Exons	The public accession number(s) of the exon(s) that are spanned by the probe	2



Field name	Description of content	Example
NCBI Gene Reference	The NCBI transcript identification number that corresponds to the gene	NM_001254.3, U77949.1, AF022109.1, CR598029.1, BC025232.1
NCBI SNP Reference	Not applicable to TaqMan [®] Gene Expression Assays.	N/A
Medline Reference	PubMed references for the gene	N/A
Celera ID	The unique Celera Discovery System (CDS) assay identification number for the gene	hCT1643963.2, hCT2294847.0
Cytogenetic Band	The chromosomal band where the gene is located. If unavailable, then the chromosome number is provided.	17q21.3
SNP Type	Not applicable to TaqMan [®] Gene Expression Assay.	N/A
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		

3

Prepare Your Samples

This chapter covers:

Prepare Your Samples	32
Store the cDNA Samples	34



Prepare Your Samples

Isolate total RNA from your samples (this page)			Evaluate the RNA (page 33).	•	Perform reverse transcription (RNA to cDNA) (page 34).
	Note: W Preparing	hen y Yo	preparing your samples, see the pr ur Samples" on page 60.	odu	act part numbers and sizes under "For
RNA Template Guidelines	For optim following • Betw • Less • Free • Diss • Free • Note the r nece was Bios • None	al I characteristics in the of of of even ssar pur ysto den : If	performance, Applied Biosystems is aracteristics: in 0.002 and 0.2 μ g/ μ L in concentration 0.005% of genomic DNA by we inhibitors of reverse transcription a ed in PCR-compatible buffer RNase activity f you suspect that the RNA contain rse transcription reaction at a final ry to add RNase inhibitor to the rev ified using the ABI PRISM [®] 6100 N ems nucleic acid purification reage atured	tior ght nd s R con vers Vuc nts.	ommends using RNA with the h of RNA PCR Nase activity, add RNase inhibitor to icentration of 1.0 U/μL. It is not e transcription reaction if the RNA leic Acid PrepStation and Applied . Denaturation of the RNA my reduce
Isolate Total RNA	To isolate TaqMan [®] www.am Note: Th use this k Ambion For other Decision www.am	e tot Ge bio e C it, i RN rec Tre bio	al RNA from cellular samples, Appene Expression Cells-to- C_T^{TM} Kit. F n.com. ells-to- C_T Kit includes the reverse to t is not necessary to evaluate the R A Isolation Decision Tree ommendations on isolating total R e: n.com/techlib/trees/RNA/index.ht	olie or p tran NA	d Biosystems recommends the procedures, refer to: scription (RNA to cDNA) step. If you (page 33).



Evaluate the RNA Use high-quality RNA that is free of contaminants (for example, proteins or detergents). You can:

- Measure the UV absorbance (A_{260}/A_{280}) to determine both quality and quantity.
- Run an agarose gel to control the quality of 18S and 28S RNA.
- Measure the RNA Integrity Number (RIN).

Detection of genomic DNA During Amplification

When you perform the experiment (Chapter 4), the TaqMan[®] Gene Expression Assay used in the TaqMan Array card may or may not detect genomic DNA. The suffix of the assay ID indicates the assay placement (and thus, potential genomic DNA interference), as described below.

Suffix	Description				
m	The assay's probe spans an exon junction. The assay does not detect genomic DNA.				
_S	The assay's primers and probe are designed within a single exon. The assay detects genomic DNA.				
	Note: For _s assays, Applied Biosystems recommends that you take precautions to exclude genomic DNA from your RNA samples. You can perform an RT-minus experiment with the TaqMan Gene Expression Assay to test for genomic DNA contaminants.				
_g	The assay may detect genomic DNA. The assay's probe may span an exon junction <i>or</i> the assay's primers and probe may be within a single exon.				
	Note: For _g assays, Applied Biosystems recommends that you take precautions to exclude genomic DNA from your RNA samples. You can perform an RT-minus experiment with the TaqMan Gene Expression Assay to test for genomic DNA contaminants.				
_mH	The assay detects transcripts belonging to a gene family with high sequence homology. The assay provides between 10 C_T and 15 C_T difference between the target gene and the gene with the closest sequence homology. Therefore, the assay detects the target transcript with 1000- to 30,000-fold greater discrimination (sensitivity) than the closest homologous transcript, if both transcripts are present at the same copy number in a sample.				
_sH					
_gH					
	• The _gH assay may detect genomic DNA.				
	The _ <i>mH</i> assay does not detect genomic DNA. The _ <i>nH</i> assay does not detect genomic DNA.				
	The _SH assay detects genomic DNA.				
_u	The assay's amplicon spans an exon junction, and the probe sits completely in one of the spanned exons. The assay does not detect genomic DNA.				
_ft	The assay detects fusion transcripts that result from chromosomal translocation. The probe and one primer are on one side of the fusion transcript breakpoint; the second primer is on the other side of the fusion transcript breakpoint. The assay does not detect genomic DNA.				
_at	The assay is designed to detect a synthetic RNA transcript with a unique sequence that lacks homology to currently annotated biological sequences.				



Perform Reverse Transcription

To perform reverse transcription (convert total RNA to cDNA), Applied Biosystems recommends one of the kits listed below. For information, refer to the applicable kit protocol.

Kit	Considerations	Protocol		
High Capacity RNA-to-cDNA Kit	 Reaction components are premixed (2 tubes), allowing for fewer pipetting steps: 20X Enzyme Mix, containing Mul V 	High Capacity RNA-to- cDNA Kit Protocol		
	Reverse Transcriptase and RNase Inhibitor Protein			
	 2X RT Buffer, containing dNTP Mix, oligo d(T) primers, and random primers 			
	Short reaction time (0.5 to 1 hour)			
High Capacity RNA-to-cDNA Master Mix	 A single tube of reagents streamlines the workflow and reduces experimental variability 	High Capacity RNA-to- cDNA Master Mix Protocol		
	 5× master mix. The RNA can be up to 80% of the final reaction volume. 			
High Capacity cDNA Reverse	Reaction components are individually available (4 tubes):	High-Capacity cDNA Reverse Transcription		
Transcription Kit	10× RT Buffer	Kits Protocol		
	• 25× dNTP Mix			
	10× RT Random Primers			
	MuLV Reverse Transcriptase			

IMPORTANT! When performing reverse transcription:

- Do not exceed the amounts of total RNA recommended in the protocol.
- To avoid introducing a 3' bias and adversely affecting the TaqMan Array card results, do not use any RT kits that use oligo d(T) primers exclusively.

Store the cDNA Samples

If you will not be using the cDNA samples immediately, store the samples at -15 to -25 °C.

To minimize repeated freeze-thaw cycles of cDNA, Applied Biosystems recommends that you store your cDNA samples in aliquots.

4

Perform the Experiment

This chapter covers:

Prepare the TaqMan [®] Array Micro Fluidic Card	36
Run the TaqMan [®] Array Micro Fluidic Card	46
Analyze the Experiment	50



Prepare the TaqMan[®] Array Micro Fluidic Card

Prepare the PCR reaction mix (cDNA + master mix) (this page).	•	Fill the TaqMan Array card with the reaction mix (page 37).	•	Centrifuge the TaqMan Array card (page 39).	•	Seal the TaqMan Array card (page 42).		
	No t unc	te: When preparing the Ta ler "For Preparing the Taql	qM <mark>Mar</mark>	an Array card, see the proc [®] Array Micro Fluidic Ca	duct rd"	part numbers and sizes on page 61.		
Recommended Amounts of cDNA	For	 the amplification (PCR) r 30 to 1000 ng (0.3 to 10 reservoir. The amount to and the number of target can use: 1000 ng (10 ng/μL) However, the cDNA quality cDNA witho 100 to 200 ng per fi 	eac ng/j use cop per co put i	tions, Applied Biosystems µL) of cDNA (converted find the depends on the expression bies per well that you need fill reservoir to detect ger ncentration will be high (1 inhibitors. eservoir to detect genes wi	rec rom n le to c nes 0 ng th n	ommends that you use: total RNA) per fill vel of your target genes detect. For example, you with low expression. g/µL); be sure to use high- noderate expression.		
	 30 to 50 ng per fill reservoir to detect genes with moderate to high expression The same amount of cDNA sample for all reactions. 							
Prepare the PCR Reaction Mix Image: WARNING CHEMICAL HAZARD. TaqMan® Universal PCR Master cause eye and skin irritation. Exposure may cause discomfort if swallowed or in Read the MSDS, and follow the handling instructions. Wear appropriate protect eyewear, clothing, and gloves. Image: WARNING CHEMICAL HAZARD. TaqMan® Gene Expression Master harmful if swallowed. Causes eye, skin, and respiratory tract irritation. Read M Safety Data Sheet and follow the handling instructions. Wear protective eyewer clothing, and gloves and use with adequate ventilation.						al PCR Master Mix may f swallowed or inhaled. propriate protective		
						xpression Master Mix is ritation. Read Material rotective eyewear,		

1. For each sample, determine the total number of reservoirs to be filled, based on the format of your TaqMan Array card.



2. Per the table below, calculate the total volume required for each reaction component:

volume for 1 fill reservoir \times the total number of fill reservoirs per sample

Include 12.5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Reaction Component	Volume (µL) for 1 Fill Reservoir
cDNA sample (30 to 1000 ng [‡]) + nuclease-free water	50.0
TaqMan® Gene Expression Master Mix	50.0
or	
TaqMan [®] Universal PCR Master Mix (with or without AmpErase [®] UNG)	
Total Volume	100.0

‡ Each sample-specific PCR reaction mix should contain 30 to 1000 ng (0.3 to 10 ng/μL) of total RNA converted to cDNA. The amount of cDNA to add depends upon the abundance of the specific gene transcript.

- **3.** If frozen, thaw the cDNA samples on ice. Resuspend the cDNA samples by inverting the tube, then gently vortexing.
- 4. Mix the master mix thoroughly by swirling the bottle.
- **5.** For each sample, label a 1.5-mL microcentrifuge tube, then add the required components to the labeled tube.
- **6.** Cap the microcentrifuge tubes, then gently vortex the tubes to thoroughly mix the solution.
- 7. Briefly centrifuge the tubes to spin down the contents and eliminate air bubbles.

Fill the TaqMan Array Card

Guidelines to ensure optimal PCR performance

- Do not remove a TaqMan Array card from its packaging until the packaging has reached room temperature and you are ready to fill it with sample-specific PCR mix. Prolonged exposure to indoor lighting can photo-degrade the fluorescent probes contained within the card. Do not expose the card to sunlight.
- Fill each fill reservoir with sample-specific PCR mix made from a single cDNA sample.
- Add 100 μ L of the sample-specific PCR mix per fill reservoir to ensure adequate filling. Volumes smaller than 100 μ L will result in insufficiently filled cards.
- Do not add sample after centrifuging the cards. When you centrifuge the card, the sample-specific PCR mix resuspends the dried TaqMan[®] probes and primers within the wells of the card. Adding sample after centrifuging disrupts the resuspended assay positions.
- To ensure a high degree of reproducibility, Applied Biosystems recommends scheduling your runs so that each card is run as soon as possible. After sealing, there is no measurable well-to-well contamination for up to 64 hours.
- Applied Biosystems recommends that you run replicate PCR reactions. Refer to the *TaqMan[®] Gene Expression Master Mix Protocol*, Appendix B.

Fill the TaqMan Array card

1. Allow the TaqMan Array card to reach room temperature, then carefully remove it from its packaging.

Note: A minimum of 15 minutes at room temperature is required to equilibrate the card.

- 2. Place the TaqMan Array card on a lab bench, with the foil side down.
- **3.** Load 100 μ L of the desired sample-specific PCR reaction mix into a 100- μ L micropipette.
- 4. Hold the micropipette in an angled position and place the tip in the fill port.

Note: There is a fill port on the left arm of each fill reservoir; it is the larger of the two holes.



IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

5. Dispense the sample-specific PCR reaction mix so that it sweeps in and around the fill reservoir toward the vent port.

IMPORTANT! Pipette the entire 100 μ L into the fill reservoir. Be careful when pushing the micropipette plunger to its second stop position (to expel the sample-specific PCR reaction mix from the tip). If a large amount of air is released, it can push the reaction mix out of the fill reservoir via the vent port or introduce bubbles into the fill reservoir.



Centrifuge the TaqMan Array Card

After the fill reservoirs have been filled with the sample-specific PCR reaction mix, centrifuge the TaqMan Array card to distribute the reaction mix to the reaction wells.

IMPORTANT! You must use a Sorvall[®] or Heraeus centrifuge with the Sorvall/Heraeus Custom Buckets and card holders. The Custom Buckets and card holders are custommade for the TaqMan Array cards. Do not use any other centrifuge or bucket/card holder system for this procedure. For more information, refer to the user manual for your centrifuge.

Note: The Sorvall and Heraeus centrifuges have either a touchpad control (EASYSet models) or knob-operated control panel (QUIKSet models). Both centrifuges use the same bucket type and card holder.

- 1. Place TaqMan Array cards into the Sorvall/Heraeus buckets:
 - a. Obtain an empty Sorvall/Heraeus Custom Bucket and card holder.

Note: The centrifuge holds four Sorvall/Heraeus buckets. Each bucket holds up to three TaqMan Array cards (loaded and/or blank balance cards) in the card holder. The card holder supports the TaqMan Array card during centrifugation.

- **b.** Place the bucket on a lab bench, with the label facing you.
- c. Insert TaqMan Array cards into the card holder, making sure that:
 - The fill reservoirs project upwards out of the card holder.
 - The reaction wells face the same direction as the "This Side Out" label.
 - You use blank balance cards to fill any open positions in the card holder. Use the blank balance cards provided with the installation kits.

IMPORTANT! Be sure to use the blank balance cards to fill any open positions. The blank balance cards will balance the centrifuge and prevent damage to the card holder. If the card holder is not completely filled, the TaqMan Array card may become displaced during centrifugation, resulting in uneven filling.



d. Place a filled card holder in the bucket so that the "This Side Out" label faces the front of the bucket.



- **2.** Set the centrifuge:
 - **a.** Power on the centrifuge.
 - **b.** Use the front panel controls to set the bucket type to **15679**.

IMPORTANT! To ensure that the maximum rotational speed stays within the manufacturer's specified limits, be sure to set the correct bucket type.

c. Use the front panel controls to set the following operations parameters:

Parameter	EASYSet (touchpad)	QUIKSet (knob-operated)	
Up ramp rate	9	3	
Down ramp rate	9	N/A	
Rotational speed	1200 rpm (331 × g)	1200 rpm	
Centrifugation time	2 × 1 min	2 × 1 min	





- **3.** Place the buckets into the centrifuge:
 - a. Press the **Open** button on the centrifuge to open the centrifuge cover.



b. Place each loaded bucket onto an open rotor arm of the centrifuge. Make sure each bucket can swing easily within its slotted position on the rotor arm.

IMPORTANT! The manufacturer recommends running the centrifuge with all four buckets. If the buckets are not fully loaded with TaqMan Array cards containing the sample-specific PCR reaction mix, place blank balance cards and card holders into the buckets. Make sure the buckets and their contents are balanced; opposing buckets should have matching weights.



- c. Close the centrifuge cover.
- 4. Run the centrifuge:
 - **a.** Press the **Start** button. The centrifuge starts, then automatically stops after 1 min, per the programmed sequence.



b. Repeat step a so that the TaqMan Array cards are centrifuged for a total of two consecutive, 1-minute spins.

IMPORTANT! To ensure complete distribution of the PCR reaction mix, you must centrifuge the TaqMan Array cards for a total of two consecutive, 1-minute spins.

- 5. Remove the TaqMan Array cards:
 - a. Press the Open button.
 - **b.** When the cover has fully opened, remove the buckets from the centrifuge, then remove the card holders from the buckets.
 - **c.** Remove all TaqMan Array cards from the buckets by gently lifting them by their carrier sides.
- **6.** Examine the TaqMan Array cards to be sure filling is complete. The amount of PCR reaction mix remaining in the fill reservoirs should be consistent from reservoir to reservoir.

Seal the TaqMan
Array CardThe TaqMan® Array Micro Fluidic Card Sealer isolates the wells of a TaqMan Array card
after it is loaded with PCR reaction mix. The sealer uses a precision stylus assembly
(carriage) to seal the main fluid distribution channels of the TaqMan Array card.

IMPORTANT! Proper operation of the sealer using a slow, steady, and deliberate motion is critical to the successful use of the TaqMan Array card.



- **1.** Position the sealer:
 - **a.** Place the sealer on a sturdy lab bench, approximately waist high so that it can be easily used.
 - **b.** Turn the sealer so that the front end (the "starting position" shown below) is closest to you and the back end is farthest from you. In the correct position, the arrows on the sealer are pointing away from you.



c. Place the sealer's carriage in its starting position.

IMPORTANT! Never insert a TaqMan Array card into the sealer if the carriage is not in its starting position. The TaqMan Array card will be irreparably damaged if the carriage is moved across it toward its starting position.

- 2. Insert a TaqMan Array card into the sealer:
 - **a.** Orient the TaqMan Array card in the proper direction over the sealer's insert plate. The card's fill reservoir end should be the end closest to the arrows etched in the base of the sealer.
 - **b.** Line up the card's rear pin grooves, foil side up, to the stylus pins on the sealer.



- **c.** Gently place the card on top of the insert plate and ensure that the front end of the card is held securely in place by the spring clips.
- d. Gently push the card until it is seated securely in the insert plate.

Note: When properly seated, the TaqMan Array card's foil surface should be level with the base of the sealer. The four spring clips ensure that the card is held in the proper position.

3. Push the carriage across the base of the sealer in the direction of the arrows. Use a slow, steady, and deliberate motion to push the carriage across the entire length of the TaqMan Array card until the carriage reaches the mechanical stops. It is important to avoid moving the carriage rapidly across the card.

CAUTION The sealer has mechanical stops at both ends to prevent the carriage from coming off. Therefore, do not use excessive force or speed when pushing the carriage.



IMPORTANT! Do not move the carriage back before removing the TaqMan Array card.

4. Remove the sealed TaqMan Array card by grasping its sides and lifting it off the sealer's insert plate. In the middle of the sealer's insert plate, there is a thumb slot to help you easily access one side of the card.





- **5.** Inspect the TaqMan Array card for proper sealing. The indentations from the stylus assembly should match up with the card's main channels. If the indentations do not match up or if the foil is in any way damaged, do not use the TaqMan Array card.
- **6.** Using scissors, trim the fill strip from the TaqMan Array card. Use the edge of the card's carrier as a guide.

CAUTION PHYSICAL INJURY HAZARD. Take care when trimming off the fill strip. Use scissors rather than razor blades or other unprotected cutting devices.





Run the TaqMan[®] Array Micro Fluidic Card

In the SDS software, set up the experiment (SDS plate document), using the SDS Setup File included on the Array Information CD (this page).	•	Perform the run on a 7900HT Fast System (page 48).

Note: When running the TaqMan Array card, see the product part numbers under "For Running and Analyzing the TaqMan[®] Array Micro Fluidic Card" on page 62.

About Running Multiple Cards After the TaqMan Array cards have been loaded and sealed, they are stable for at least 64 hours. Therefore, for high-throughput TaqMan Array card processing, you can use the Automation Accessory. For information on the Automation Accessory, refer to the *Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database* User Guide.

Set Up the SDS1. SPlate Document2

1. Start SDS Software v2.1 or later.

2. Select File → New.

- **3.** Complete the New Document dialog box:
 - a. From the Assay dropdown menu, select $\Delta\Delta C_T$ (RQ) or Relative Quantification.
 - b. From the Container dropdown menu, select **384 Wells TaqMan Low Density** Array.
 - c. Complete the remaining fields as shown, then click OK.

New Doc	cument 🛛	The Assay field selections
Assay	: ДАСТ (RQ)	vary, depending on your software version.
Container	: 384 Wells Taqman Low Density Array	
Template	: Blank Template	
	Browse	
Barcode	×	_ (Recommended) Click the
?	Save Settings As My Default OK Cancel	type the barcode.

- 4. Import the SDS Setup File (*.txt) into the new SDS plate document:
 - **a.** In the CD drive, insert the Array Information CD that shipped with your TaqMan Array card.
 - **b.** In the SDS software, select **File** > **Import**.



c. In the Import dialog box, navigate to the SDS Setup File for your TaqMan Array card, then click **Import**. The SDS software imports information from the SDS Setup File into the SDS plate document.

Note: The SDS software uses the information from the SDS Setup File to automatically configure the plate grid and setup table with detector, detector task, marker, and sample data. For details on the SDS Setup File, see page 26.

IMPORTANT! Modifying the contents of the SDS Setup File can corrupt the file, making the file unusable (that is, you will not be able to access information for the TaqMan Array card).

🗵 Import	×	
Look in:	🔁 Custom TaqMan Low Density Array 💿 🖻 📺 🧱	
📕 Sample TL	DA Setup File txt	
File name:	Sample TLDA Setup File.txt Import	
Files of type:	Tab-delimited Text (*.bd)	

- 5. Save the SDS plate document:
 - a. Select File ► Save As.
 - **b.** Navigate to a save location.
 - c. Enter a name for the SDS plate document.
 - d. For Files of Type, select SDS 7900HT Document (*.sds) or SDS 7900HT Template Document (*.sdt).

Note: You can save the plate document as an SDS plate document (*.sds) or SDS template (*.sdt). Saving the plate document as an SDS template is recommended when you want to create duplicate plate documents for a series of TaqMan Array cards with identical assay configurations. For more information on SDS templates, refer to the *SDS Online Help*.

e. Click Save.

Perform the Run A TaqMan[®] Array Micro Fluidic Card Thermal Cycling Block must be installed on the instrument. For more information, see "Instrument Overview" on page 5.



- **1.** Open the SDS plate document in the SDS software.
- 2. Select the Instrument tab.
- **3.** Use the default thermal cycling conditions, as shown in the Thermal Cycler tab.

Note: When you selected 384 Well TaqMan Low Density Array (step 3 on page 46), the SDS software automatically set the appropriate thermal cycling conditions for the TaqMan Array cards.

- 4. Select the **Real-Time** tab, then verify that **Ell** Connected to 'PlateName') is displayed in the status bar. If the software is not connected to the instrument, click **Connect to Instrument**.
- 5. If the instrument tray is inside the instrument, click **Open/Close** to rotate the instrument tray to the OUT position.
- **6.** Verify that the TaqMan Array Micro Fluidic Card Thermal Cycling Block is installed in the instrument tray. If the block is not installed, you must:
 - Remove the existing block.
 - Install the TaqMan Array Micro Fluidic Card Thermal Cycling Block.
 - Change the plate adapter. For detailed information on changing the instrument blocks, refer to the *Applied Biosystems 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide*.
- 7. Place the prepared TaqMan Array card in the instrument tray with:
 - Well A1 at the top left corner of the tray and the notched corner at the top right.
 - The barcode toward the front of the instrument.



- 8. Click Start Run. The instrument tray rotates to the IN position. During the run, the instrument displays real-time status information in the Instrument → Real-Time tabs and records the fluorescence emissions. During the run, you can view the data (as the data are generated in real-time).
- **9.** When the Run Complete dialog box appears, click **OK** to close the dialog box, click **Open/Close**, then remove the TaqMan Array card from the instrument tray.



Analyze the Experiment





3. Review the gene expression plot (for SDS Software v2.3, view the amplification plots in the Plate, Detector, or Sample view).

Note: Relative gene expression measurements within and across manufacturing lots of TaqMan Array cards are reproducible. You can compare normalized data (ΔC_T values), but not raw C_T values.

IMPORTANT! If you are using different lots of TaqMan Array cards with the same set of assays, be sure to use the correct SDS Setup File. Different lots with the same set of assays may ship with different SDS Setup Files.

(Optional) Perform Downstream Analysis

For further data analysis, the raw C_T and the ΔC_T values can be exported. If you are using SDS Software v2.3, you can export the values from the Plate Centric view.

For detailed downstream analysis, Applied Biosystems recommends the Real-Time StatMiner[™] Software. For information, refer to:

www.integromics.com/StatMiner.php





Troubleshooting

The following table lists some possible errors, possible causes, and recommended actions.

Observation	Possible Cause	Recommended Action		
After removing the TaqMan [®] Array Micro Fluidic Card from its packaging				
Water condenses on the reaction wells (optical side of the TaqMan Array card).	The TaqMan Array card may not have come to room temperature before being removed from its packaging.	Remove condensation by lightly blowing on the reaction wells. You can use room temperature pressurized nitrogen or an air blower.		
		IMPORTANT! Be sure to remove all water condensation. The exterior surface of the reaction wells (optical side of the TaqMan Array card) must be free of water condensation.		
After pipetting				
Too little PCR reaction mixture has gone into the fill reservoir.	The PCR mixture was not correctly pipetted into the fill reservoir.	Take care to correctly pipette the entire PCR reaction mixture (100 μ L) into the fill resonance.		
Some of the PCR reaction mixture leaks out of the vent port in the fill reservoir.		ni reservoir. Add more sample.		
Bubbles introduced into fill ports.	Air was introduced from pushing the pipette plunger in to its second stop position.	Inspect the affected wells after centrifuging and sealing the TaqMan Array card. Note wells that contain bubbles, and delete these wells from analysis.		



Observation	Possible Cause	Recommended Action		
After centrifuging				
(Rare) Reaction mix remains in a fill reservoir.	The fill port is blocked (the fill reservoir is defective).	Inspect the TaqMan Array card for a blocked fill port or a pinched channel. If the fill reservoir is defective, contact Applied Biosystems Technical Support.		
	Filling is incomplete or not consistent.	Centrifuge the TaqMan Array card again for 1 minute. If the reservoir filling is still not complete after the additional		
Excess reaction mix in a fill reservoir		 For complete after the additional centrifuge cycle, and you choose to process the TaqMan Array card further, you should void the results for the affected fill reservoir (sample). IMPORTANT! Do not exceed 1200 rpm or accumulated centrifugation times of more than 3 minutes. Excessive centrifugation speeds and times may deform the TaqMan Array card. 		
(Rare) A fill reservoir is completely Some wells were not filled properly. drained.		If you choose to process the TaqMan Array card further, you should void the results for the affected fill reservoir		
Fill reservoir is completely drained		(sample).		
After Run/Analysis				
Amplification Plots are noisy across portions of the TaqMan Array card. In the Multicomponent Plot, the FAM [™] and ROX [™] dye signals are below the background for the affected wells.	The TaqMan Array card is misaligned in the TaqMan [®] Array Micro Fluidic Card Thermal Cycling Block.	Check the TaqMan Array card for crushed or distorted feet. If there are crushed feet, contact Applied Biosystems Technical Support or contact your Applied Biosystems Field Applications Specialist (FAS).		
No amplification across large portions of the TaqMan Array card. In the Multicomponent Plot, the FAM [™] and ROX [™] dye signals are				

below the background.Empty wells due to improper staking.Use a steady, even motion when using
the staker.No amplification. The
Multicomponent Plot shows a
rapid drop in ROX™ dye signal.Empty wells due to improper staking.Use a steady, even motion when using
the staker.



Observation	Possible Cause	Recommended Action
No amplification within or across one or more rows within a single fill recenvoir. The Multicomponent	Empty wells due to improper staking.	Use a steady, even motion when using the staker.
Plot shows a rapid drop in ROX [™] dye signal.	Empty wells due to misalignment of the staker stylus.	If the staker stylus is misaligned, contact your Applied Biosystems FAS.
	No master mix loaded in the fill reservoir.	Add master mix.
No amplification or poor amplification for a specific assay.	Low-abundance gene.	Use more cDNA.Preamplify the cDNA.
The SDS v2.3 QC Flag is <i>BPR</i> (Bad Passive Reference). The Multicomponent Plot may show a gradual drop in ROX [™] dye fluorescence.	Minor leaking from the wells due to inadequate sealing.	Use a steady, even motion when using the staker.
	Bubbles in well(s).	Use proper pipetting techniques to avoid introducing bubbles when loading the fill reservoirs.
		Note: To determine if the data are usable:
		 Inspect the Multicomponent Plot. The FAM[™] and ROX[™] dye signals should be parallel.
		• Inspect the Amplification Plot. There should not be any spikes or bumps in the exponential phase where the plot crosses the threshold. Compare the C_T values to replicate wells. If the values are comparable, then the data are usable; if the values are not comparable, remove the well(s) as an outlier.



Observation	Possible Cause	Recommended Action	
Replicates have poor precision (the standard deviation value is > 0.5 for assays with a C _T value that is < 30).	Individual assay performance (rising baseline or weak amplification).	Use the manual baseline setting. Adjust the baseline and threshold settings, then reanalyze.	
	Bubbles in well(s).	Use proper pipetting techniques to avoid introducing bubbles when loading the fill reservoirs.	
		 Adjust the baseline and threshold settings, then reanalyze. 	
		Remove the replicate well(s) as an outlier.	
	Leaky well(s) due to improper staking. The QC Flag is <i>BPR</i> .	Omit the leaky well(s) from analysis, then reanalyze the data.	
		 Use a steady, even motion when using the staker. 	
	Not all of the card holder positions were filled before centrifuging.	Be sure to fill all of the card holder positions with blank TaqMan Array cards before centrifuging.	
	Non-validated centrifuge.	Use a Sorvall [®] or Heraeus centrifuge.	
	Non-AB master mix.	Use AB master mix.	
No amplification or poor precision across many assays.	Non-AB master mix.	Check the protocol; be sure to add the	
	No reagent (master mix).	sample and master mix.	
	No sample.		
	Inhibitor in the sample.		



TaqMan[®] Endogenous Control Assays

To help with normalization, you can select a human, mouse, or rat endogenous control assay from the list of inventoried endogenous controls in Table 1 below.

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.

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

Table 1 Candidate endogenous control assays for normalization (PN 4331182)


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С

Required Materials

This appendix covers:

For Preparing Your Samples	60
For Preparing the TaqMan [®] Array Micro Fluidic Card	61
For Running and Analyzing the TaqMan [®] Array Micro Fluidic Card	62
For General Use	63



For Preparing Your Samples

Reagents

Item	Size	Part Number
TaqMan [®] Gene Expression Cells-to-CT [™]	100 lysis reactions/500 PCR	AM1728
it.	400 lysis reactions/2000 PCR	AM1729
High Capacity cDNA Reverse	200 reactions	4368814
	200 reactions with RNase Inhibitor	4374966
	1000 reactions	4368813
	1000 reactions with RNase Inhibitor	4374967
High Capacity RNA-to-cDNA Master Mix	200 reactions	4390778
High Capacity RNA-to-cDNA Kit	50 reactions	4387406

Notes



For Preparing the TaqMan[®] Array Micro Fluidic Card

Reagents

Item	Size; Description	Part Number
TaqMan [®] Gene Expression Master Mix	Mini-Pack; One 1-mL tube	4370048
(2^)	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
with Amperase ² UNG (2X)	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
TaqMan [®] Universal PCR Master Mix, No	1-Pack; One 5-mL bottle	4324018
Amperase - ONG	2-Pack; Two 5-mL bottles	4364341
	5-Pack; Five 5-mL bottles	4364343
	10-Pack; Ten 5-mL bottles	4324020
	Bulk-Pack; One 50-mL bottle	4326614
TaqMan [®] Array Custom Micro Fluidic Card	One TaqMan Array card; 384 wells per card	See Chapter 2 on page 9 for
TaqMan [®] Gene Set	One TaqMan Array card; 384 wells per card	information.
TaqMan [®] Gene Signature Array	The endogenous control Gene Signature Arrays come in sets of two cards; the remaining Gene Signature Arrays come in sets of four cards. 384 wells per card.	

Equipment

Item	Part Number
Micropipettes, 100-μL	Major laboratory supplier (MLS)
Sorvall [®] or Heraeus centrifuge	Contact your local Applied
Rotor	Representative.

Notes



Item	Part Number
Sorvall/Heraeus Custom Buckets	Included in the 7900HT TaqMan [®]
Sorvall/Heraeus card holders	Upgrade (hardware upgrade kit).
TaqMan [®] Array Micro Fluidic Card Sealer	

For Running and Analyzing the TaqMan[®] Array Micro Fluidic Card

Instrument and Accessories

Item	Part Number
Applied Biosystems 7900HT Fast Real-Time PCR System	Contact your local Applied Biosystems Sales Representative.
7900HT TaqMan [®] Array Micro Fluidic Card Upgrade (hardware upgrade kit)	4329012
This kit includes:	
 TaqMan[®] Array Micro Fluidic Card Thermal Cycling Block 	
TaqMan [®] Array Micro Fluidic Card Sealer	
 Four centrifuge buckets and card holders (specific to the Sorvall[®] or Heraeus centrifuge) 	
 7900HT TaqMan[®] Array Micro Fluidic Card Chemical Installation Kit: 	
 Spectral Calibration Kit 	
 TaqMan[®] Array Micro Fluidic Card Instrument Verification RNase P Kit 	
 Calibration Cards (4 cards) 	

Software

Item	Part Number
SDS Software v2.1 or later	Contact your local Applied
Note: SDS Software v2.1 through v2.2.2 include the $\Delta\Delta C_T$ Study program. SDS Software v2.3 includes the RQ Manager program. Both programs are provided for relative quantitation analysis.	Biosystems Sales Representative.
Real-Time StatMiner [™] Software	Go to www.integromics.com/StatMiner.php.

Notes



For General Use

For more product recommendations, visit the real-time PCR decision tree:

www.appliedbiosystems.com/qpcrtree

Item	Source
Disposable gloves	MLS
Microcentrifuge	MLS
Microcentrifuge tubes, 1.5-mL	MLS
Nuclease-free Water (not DEPC-treated)	Applied Biosystems PN AM9930
Pipette tips, with filter plugs	MLS
Pipettes, positive-displacement or air-displacement	MLS
Polypropylene tubes	MLS
Tris-EDTA (TE) buffer, pH 8.0	MLS
Vortexer	MLS

Notes_



Appendix C Required Materials For General Use

Prevent Contamination

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.

Good Laboratory Practices for PCR

When preparing samples for PCR amplification:

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves.
- 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.



Notes_

Index

Numerics

7900HT Fast Real-Time PCR System 5

Α

AIF. See Assay Information File amplification no/poor amplification 55, 56 noisy amplification plot 54 analyze the TaqMan Array card 50–51 Applied Biosystems contacting vii Technical Support vii Array Information CD 26 Array Map File description 27 formats 27 Assay Information File description 27

В

balance cards 39, 41 biohazardous waste, handling xii bubbles 53

С

CAUTION, description x, 48 cDNA convert from RNA 34 recommended amounts 36 centrifuge system balance cards 39, 41 bucket requirements 39 centrifuge a TaqMan Array card 39 components 5 EASYset control panel 40 QUIKset control panel 40 TaqMan Array card, placing in bucket 39 chemical safety X chemical waste safety xii condensation 53contamination. See prevent contamination 65 conventions for describing menu commands V

IMPORTANTS! **v** Notes **v** text **v** user attention words **v**

D

DANGER, description x downstream analysis 51

Ε

EASYset control panel 40 efficiency, TaqMan Gene Expression Assay 3 excess reaction mix 54 experiment analyze 50–51 before you begin 5 review results 50

F

filling a TaqMan Array card guidelines 37 procedure 38 formats Array Map File 27 TaqMan Array card 22–25

G

genomic DNA detection 33 guidelines chemical safety x chemical waste safety xii filling a TaqMan Array card 37 prevent contamination 65 RNA template 32

Η

Help system, accessing vii

import the SDS Setup File 46 IMPORTANT, description x instruments required components 5 required to run TaqMan Array card 5

L

laboratory practices 65

Μ

materials. See required materials 59–63 MSDSs description xi obtaining vii, xi multiple cards 46

0

online Help. *See* Help system ordering TaqMan Array Custom Micro Fluidic Card 10, 19, 21 TaqMan Gene Sets 10, 19, 21 TaqMan Gene Signature Array 16

Ρ

PCR step TaqMan Array card, placing in bucket 39 poor precision 55 prevent contamination good laboratory practices for PCR 65 guidelines 65 product overview TaqMan Array card 2 TaqMan Array Custom Micro Fluidic Card 2 TaqMan Gene Expression Assay 3 TaqMan Gene Sets 2 TaqMan Gene Signature Array 2

Q

QUIKset control panel 40

R

radioactive waste, handling xii reaction mix prepare 36 troubleshooting 53, 54 Real-Time StatMiner Software 51 required materials 59–63 general use 63 running and analyzing 62 sample preparation 60 TaqMan Array card preparation 61 reverse transcription, performing on samples 34 RNA convert to cDNA 34 detect genomic DNA 33 evaluate 33 isolate total 32 template guidelines 32 run the TaqMan Array card 48 running multiple TaqMan Array cards 46

S

safety biological hazards xiii guidelines X, XII samples detect genomic DNA 33 evaluate RNA 33 isolate total RNA 32 perform reverse transcription 34 prepare 32–34 RNA template guidelines 32 store 34 SDS Plate Document setup 46 SDS Setup File description 26 import 46 using 46 seal the TaqMan Array card 42 StatMiner Software 51 store samples 34

Т

TaqMan Array card analyze the experiment 50-51applications 4 centrifuge 39 diagram 4 downstream analysis 51 fill 38 filling guidelines 37 formats 22-25 instruments required to run 5multiple cards 46 placing in bucket 39 product overview 2 reaction mix 36recommended amounts of cDNA 36 review experiment results 50run 48 seal 42 set up the SDS Plate Document 46 troubleshooting 53–56

workflow for performing experiments with 6TaqMan Array Custom Micro Fluidic Card ordering 10, 19, 21 product overview 2 TaqMan Array Micro Fluidic Card Sealer 5, 42 diagram 43 TaqMan Array Micro Fluidic Card Thermal Cycling Block 5 TaqMan Gene Expression Assay efficiency 3 product overview 3 TaqMan Gene Sets ordering 10, 19, 21 product overview 2 TaqMan Gene Signature Array ordering 16 product overview 2 Technical Support, contacting vii training, information on vii troubleshooting 54 bubbles 53 condensation 53 drained fill reservoir 54 no/poor amplification 55, 56 noisy amplification plot 54 poor precision 55 reaction mix leaks 53 too little reaction mix 53

U

user attention words, described V

W

WARNING, description x waste disposal, guidelines xii workflow 6 Index

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