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Custom TaqMan® PreAmp Pools

Early Access

Protocol

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Contents

About This Guide

Purpose

The *Custom TaqMan*® PreAmp Pools Protocol provides procedural and reference information for the Custom PreAmp Pools.

Each Custom TaqMan PreAmp Pool contains preamplification oligonucleotides for your targets of choice.

Note: PreAmp Pools do not contain an assay for 18S RNA.

Safety information

Note: For general safety information, see this section and "Safety" on page 39. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

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

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

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

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

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

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 41.

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

Custom TaqMan® PreAmp Pools

Product information

Purpose of the product

The Custom TaqMan® PreAmp Pools are prepooled sets of TaqMan Gene Expression Assays that are to be used with compatible TaqMan Arrays (TaqMan Array Cards and TaqMan Array Plates). The PreAmp Pools are intended for use with:

- Very small quantities of sample RNA
- Genes that have low expression levels

Use the PreAmp Pools to increase the quantity of your sample targets. You can directly compare preamplified samples using the Custom TaqMan PreAmp Pools without introducing bias.

Note: If you want to directly compare unamplified samples to preamplified samples, Applied Biosystems recommends that you perform a uniformity experiment. See Appendix B on page 29.

Storage

Store the PreAmp Pools at –15 to –25 °C, protected from light.

Required materials, equipment, and software

For sample preparation

Reagents

Product	Size	Part number
RNAqueous®-Micro Kit	50 purifications	AM1931
TaqMan Gene Expression Cells-to-CT™ Kit	100 lysis reactions/500 PCR	AM1728
	400 lysis reactions/2000 PCR	AM1729
RecoverAll™ Total Nucleic Acid Isolation Kit	40 purifications	AM1975
High Capacity cDNA Reverse Transcription Kit	200 reactions	4368814
	200 reactions with RNase Inhibitor	4374966
	1000 reactions	4368813
	1000 reactions with RNase Inhibitor	4374967
High Capacity RNA-to-cDNA Kit	50 reactions	4387406

For preamplification

Reagents

Product	Size/Description	Part number
TaqMan PreAmp Master Mix (2X)	40 preamplification reactions, 120 µL each	4391128
Custom TaqMan PreAmp Pools	250 preamplification reactions, 7.5 µL (TaqMan cards) 600 preamplification reactions, 7.5 µL (TaqMan plates)	4441856

Equipment

Instrument	Part number
GeneAmp® PCR System 9700 7500 Real-Time PCR System Veriti 96-Well Fast Thermal Cycler	Contact your local Applied Biosystems sales representative.

Reaction tubes and plates

Item	Source	Part number
MicroAmp® Reaction Tube with Cap, 0.2-mL	Applied Biosystems	N8010540
MicroAmp 8-Tube Strip, 0.2-mL (125 strips)		N8010580
MicroAmp 8-Tube Strip, 0.2-mL (120 strips, assorted colors)		N8010838
MicroAmp 8-Cap Strip (300 strips)		N8010535
MicroAmp 8-Cap Strip (300 strips, assorted colors)		N8010835
MicroAmp Fast Optical 96-Well Reaction Plate with Barcode (200 plates)		4366932
MicroAmp Optical Adhesive Film (100 films)		4311971
MicroAmp Clear Adhesive Film (100 films)		4306311
MicroAmp Optical Film Compression Pad (5 pads)		4312639

For amplification using a TaqMan® Array Micro Fluidic Card or TaqMan® Array Plate

Reagents

Product	Size/Description	Part number
TaqMan Array Micro Fluidic Cards	Format 12	4342247
	Format 16	4346798
	Format 24	4342249
	Format 32	4346799
	Format 48	4342253
	Format 64	4346800
	Format 96a	4342259
	Format 96b	4342261
	Format 192	4346802
	Format 384	4342265

Product *(continued)*	**Size/Description**	**Part number**
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
TaqMan Universal Master Mix II (no UNG)	Mini Pack; one 1-mL tube	4440043
	1-Pack; one 5-mL bottle	4440040
	2-Pack; two 5-mL bottles	4440047
	5-Pack; five 5-mL bottles	4440048
	10-Pack; ten 5-mL bottles	4440049
	Bulk-Pack; one 50-mL bottle	4440041
TaqMan Universal Master Mix II (with UNG)	Mini Pack; one 1-mL tube	4440042
	1-Pack; one 5-mL bottle	4440038
	2-Pack; two 5-mL bottles	4440044
	5-Pack; five 5-mL bottles	4440045
	10-Pack; ten 5-mL bottles	4440046
	Bulk-Pack; one 50-mL bottle	4440039
TaqMan Universal PCR Master Mix (with AmpErase® UNG)	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
TaqMan Universal PCR Master Mix (no AmpErase UNG)	1-Pack; one 5-mL bottle	4324018
	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

Plates

Item	**Part number**
TaqMan Array Standard 96 well Plates	Std_96
TaqMan Array Fast 96 well Plates	Fast_96

Equipment

Item	Part number
StepOne™ System StepOnePlus™ System 7300 System 7500 System 7500 Fast System 7900HT Fast System	Contact your local Applied Biosystems sales representative.
7900HT TaqMan Array Upgrade (hardware upgrade kit). This kit includes: TaqMan Array Thermal Cycling Block TaqMan Array Sealer Four centrifuge buckets and array holders (specific to the centrifuge) 7900HT TaqMan Array Chemical Installation Kit: Spectral Calibration Kit TaqMan Array Instrument Verification RNase P Kit Calibration Cards (4 cards)	4329012
Sorvall®, Heraeus, SL 40, or Rotanta centrifuge	Contact your local Applied Biosystems sales representative.

Software

Item	Part number
Sequence Detection System (SDS) Software v2.1 or later 7500 SDS v1.4 7500 v2.0 StepOne Plus v2.1 **Note:** SDS Software v2.1 through v2.4 include the $\Delta\Delta C_T$ Study program. **Note:** SDS Software v2.3 or later includes the RQ Manager program. Both programs are provided for relative quantitation analysis.	Contact your local Applied Biosystems Sales Representative.
DataAssist™ Software	Go to **www.appliedbiosystems.com/dataassist**.
RealTime StatMiner® Software	Go to **www.integromics.com/StatMiner.php**.

For general use

The following materials and equipment are required for using Custom TaqMan PreAmp Pools. Unless otherwise noted, many items listed are available from major laboratory suppliers (MLS).

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

‡ For the Safety Data Sheet of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Workflow

Prepare your samples (page 14)

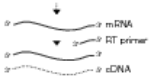
Isolate the total RNA

For recommendations, refer to the Ambion RNA Isolation Decision Tree: **www.ambion.com/techlib/trees/RNA/index.html**

Evaluate the total RNA

Perform reverse transcription

(Optional) Evaluate the cDNA



High Capacity RNA-to-cDNA Kit
or
High Capacity cDNA Reverse Transcription Kit

Perform the preamplification reactions (page 16 and page 22)

Prepare the preamplification reactions for a TaqMan® Array Micro Fluidic Card

or

Prepare the preamplification reactions for a TaqMan® Array Plate

Perform thermal cycling

A thermal cycler

Perform the PCR amplification reactions (page 19 and page 23)

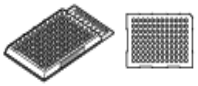
Prepare the reaction mix

A TaqMan master mix

Prepare the TaqMan® Array Micro Fluidic Card

or

Prepare the PCR amplification reactions for a TaqMan® Array Plate



TaqMan Array Micro Fluidic Card or TaqMan Array Plate

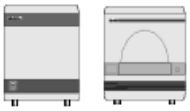
Set up the SDS plate document

System software and information CD

Perform qPCR on a TaqMan® Array Micro Fluidic Card

or

Perform qPCR on a TaqMan® Array Plate



Real-Time PCR System

Analyze the experiment (page 25)

Review the data

Perform downstream analysis

Prepare your samples

Isolate total RNA from your samples (page 15).	Evaluate the total RNA (page 15).	Perform reverse transcription (RNA to cDNA) (page 16).	(Optional) Evaluate the cDNA (page 16).

When preparing your samples, see page 8 for product part numbers and sizes.

RNA template guidelines

For optimal performance of the reverse transcription kit, master mixes, and gene expression assays, Applied Biosystems recommends using RNA that is:

- Between 0.002 and 0.2 µg/µL in concentration
- <0.005% of genomic DNA by weight
- Free of inhibitors of reverse transcription and PCR
- Dissolved in PCR-compatible buffer

- Free of RNase activity

  Note: If you suspect that the RNA contains RNase activity, add RNase inhibitor to the reverse transcription reaction at a final concentration of 1.0 U/µL. If the RNA is purified using the ABI Prism® 6100 Nucleic Acid PrepStation and Applied Biosystems nucleic acid purification reagents, do not add RNase inhibitor to the reverse transcription reaction.

- Nondenatured

  Note: Do not denature the RNA. Denaturing the RNA may reduce the yield of cDNA for some gene targets.

Isolate the total RNA

To isolate the total RNA from your samples, Applied Biosystems recommends one of the kits listed below. For information on these kits, refer to:

www.ambion.com

Starting Material	Kit	Part Number
Limited amounts of cellular material	TaqMan Gene Expression Cells-to-CT™ Kit‡	AM1728 AM1728M AM1729 AM1729M 4399002 4399002M
Limited amounts of non-cellular material	RNAqueous®-Micro Kit	AM1931
Formaldehyde- or paraformaldehyde-fixed, paraffin-embedded (FFPE) tissue	RecoverAll™ Total Nucleic Acid Isolation Kit	AM1975

‡ Includes the reverse-transcription step (RNA-to-cDNA).

For other recommendations on isolating total RNA, refer to the Ambion RNA Isolation Decision Tree:

www.ambion.com/techlib/trees/RNA/index.html

Evaluate the total RNA

Use high-quality RNA that is free of contaminants (proteins, detergents, and so on). You can:

- Measure the UV absorbance (A260/A280) to determine both quality and quantity.
- Measure the RNA integrity number (RIN).
- Run an agarose gel to determine the quality of 18S and 28S RNA.

Note: Very small amounts of sample may not produce enough RNA to evaluate quality. In these cases, Applied Biosystems recommends that you use a reliable extraction method (see the table above in the "Isolate the total RNA" section).

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

(Optional) Evaluate the cDNA

Applied Biosystems recommends UV absorbance (A260/A280) measurements to evaluate the cDNA.

Note: If cDNA is from a single cell, assume 15 to 25 picograms of cDNA are present. Applied Biosystems does not recommend quantitation for single cells.

Prepare the preamplification reactions for a TaqMan® Array Micro Fluidic Card

Prepare the preamplification reactions (this page).	Perform thermal cycling (page 18).

When performing the preamplification reactions, see page 8 for product part numbers and sizes.

Recommended amounts of cDNA

For the preamplification reactions, Applied Biosystems recommends that you use:

- 0.2 to 100 ng of cDNA per reaction

  Note: Applied Biosystems is developing specific protocols for use with single-cells. For more information, refer to Appendix C on page 35.

- The same amount of cDNA for all reactions

Prepare the preamplification reactions

Reaction volumes will differ according to the format of TaqMan Array Micro Fluidic Cards.

Use the following table to determine the number of fill reservoirs to load on the TaqMan Array Micro Fluidic Card.

	TaqMan Array Card Format									
	12	**16**	**24**	**32**	**48**	**64**	**96a**	**96b**	**192**	**384**
Number of fill reservoirs loaded per sample	1	1	1	2	1	4	2	4	4	8

IMPORTANT! Prevent contamination during preamplification. Refer to Appendix A on page 27 for guidelines.

1. Determine the total number of preamplification reactions to run.

2. Per the preamplification reaction component table on page 18, calculate the total volume required for each reaction component:

   volume for 1 reaction × the total number of reactions

   Include 20% excess volume in your calculations to compensate for the loss that occurs during pipetting.

   Note: Formats 48, 96a, 192, and 384 contain fill reservoirs with singleton targets, and multiple sets of fill reservoirs must be loaded if replicates are desired.

   Note: Formats 32, 64, 96a, 96b, 192, and 384 require loading multiple fill reservoirs with the same sample in order to assay all targets. For example, 8 reservoirs on Format 384 are filled with the same sample to analyze targets in 384 wells.

   Note: More than one card is needed to run replicates of Format 384.

Use the following table to determine the economical volume of preamplification reaction needed based on the number of fill reservoirs (sufficient for loading duplicate sets of fill reservoirs).

Preamplification Reaction Component	Volume (µL)/Reaction			
Number of fill reservoirs per sample	**1**	**2**	**4**	**8**
cDNA, 0.2 to 100 ng + nuclease-free water	2	4	7.5	15
Custom TaqMan PreAmp Pool	2	4	7.5	15
TaqMan PreAmp Master Mix (2X)	4	8	15	30
Total volume	8	16	30	60

3. Thaw the TaqMan PreAmp Master Mix and Custom TaqMan PreAmp Pools on ice. Gently invert the tubes to mix, briefly spin, then return the tubes to ice.
4. In a 0.2-mL reaction tube, tube strip, or 96-well optical reaction plate, combine the required volumes of each reaction component on ice.
5. Cap the reaction tube or seal the reaction plate.
6. Invert the reaction tube or plate several times to mix, then briefly centrifuge.

Perform thermal cycling

You can thermal-cycle your preamplification reactions in any of the following instruments:

- GeneAmp PCR System 9700
- 7500 Real-Time PCR System
- Veriti 96-Well Fast Thermal Cycler

Note: Thermal cycling for preamplification requires approximately 1.5 hours.

1. Load the reaction tube or plate into the instrument.
2. Set up the run method using the following conditions:
   - Reaction volume (µL): enter volume up to **100 µL**
   - Ramp speed or mode: **Max**
   - Thermal profile:

Hold	Cycle (14 cycles)		Hold	Hold
	Denature	**Anneal/Extend**		
95 °C	95 °C	60 °C	99.9 °C	4 °C
10 min	15 sec	4 min	10 min	∞

3. After the run is complete, unload the reaction tube or plate.

STOPPING POINT Proceed directly to "Perform the PCR amplification reactions for a TaqMan® Array Micro Fluidic Card". Alternatively, you can store the preamplified product at –20 °C for up to 2 weeks.

IMPORTANT! Applied Biosystems recommends running the TaqMan Array Cards within 64 hours after loading the preamplification product. Standard TaqMan Array Plates should be run 24 hours after loading the preamplification product. Fast TaqMan Array Plates should be kept on ice until they are used.

Perform the PCR amplification reactions for a TaqMan® Array Micro Fluidic Card

Procedure

Prepare the reaction mix (preamplified cDNA + master mix) (this page).	Prepare the TaqMan Array Micro Fluidic Card (page 20).	Set up the SDS plate document (page 21).	Run the TaqMan Array on a Real-Time PCR System to perform qPCR (page 22).

Note: When performing the amplification reactions, see page 9 for product part numbers and sizes.

Prepare the reaction mix

1. If the preamplified cDNA is frozen, thaw it on ice. To resuspend the preamplified cDNA, invert the tube several times, then briefly centrifuge the tube.
2. Mix the TaqMan master mix thoroughly by swirling the bottle.

   Note: Remember to include an NTC control when planning your experiments (use nuclease-free water in place of preamp product).

3. In a microcentrifuge tube, combine the required volumes for all reaction components, per the table below. Minimum volumes needed to fill the required number of reservoirs or columns for one TaqMan Array Micro Fluidic Card are shown.

   Note: Each TaqMan Array Micro Fluidic Card reservoir requires ~100 µL volume.

TaqMan Array Micro Fluidic Card	12	16	24	32	48	64	96a	96b	192	384
Number of fill reservoirs per sample	1	1	1	2	1	4	2	4	4	8
	Volume (µL)/Reaction									
µL PreAmp Product	3	3	3	7	3	14	7	14	14	28
Nuclease-free water	52	52	52	103	52	206	103	206	206	413
µL 2X Master Mix	55	55	55	110	55	220	110	220	220	440
Total volume	110	110	110	220	110	440	220	440	440	880
Number of instances	4	3	2	3	1	3	1	2	1	1

   IMPORTANT! Adding more preamplified cDNA increases the fluorescence baseline and may result in problems with analysis. Volumes for a recommended dilution of 1:32 are shown in the table above.

4. Cap the tube, then gently vortex to thoroughly mix the solution.

5. Briefly centrifuge the tube to spin down the contents and eliminate air bubbles.

Prepare the TaqMan® Array Micro Fluidic Card

Use a TaqMan Array Micro Fluidic Card that contains the TaqMan Gene Expression Assays that target the same genes as your Custom TaqMan PreAmp Pool (with the exception of the 18S gene). For details on filling and sealing the array, refer to the *TaqMan Array Micro Fluidic Cards User Guide* (PN 4400263).

In summary:

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

   IMPORTANT! A minimum of 15 minutes at room temperature is required to equilibrate the array.

2. Add the reaction mix:

   Add 100 µL of the reaction mix (preamplified cDNA + master mix) to each fill reservoir of the TaqMan Array.

3. Centrifuge the TaqMan Array Micro Fluidic Card using a compatible centrifuge. For a list of compatible centrifuges, see the Centrifuge Compatibility list at

   http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_071141.pdf

4. Seal and trim the TaqMan Array.

Set up the SDS plate document

1. Refer to the instrument user guide for setting up the plate document. Follow the instructions on the CD that is provided with TaqMan Arrays:
   a. Open SDS Software v2.3 or later.
   b. Select **File ▸ New**.
   c. In the New Document dialog box pull-down menu, select:
      Assay: **Std Curve (AQ)**
      Container: **384 Wells TaqMan Low Density Array**
   d. Click **OK**.

2. Import the SDS Setup File (*.txt) into the new SDS plate document:
   a. In the CD drive, insert the Array Information CD that is shipped with your TaqMan Array Micro Fluidic Card or Plate.
   b. In the SDS software, select **File > Import**.
      (Recommended) Click the **Barcode** field, then scan or enter the barcode. The Assay field selections vary, depending on your software version.
   c. In the Import dialog box, navigate to the SDS Setup File for your array, then click **Import**. The SDS software imports information from the SDS Setup File into the 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.

      CAUTION! Modifying the contents of the SDS Setup File can corrupt the file, making the file unusable. You will not be able to access information for the TaqMan Array.

3. 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 Arrays that have identical assay configurations. For more information on SDS templates, refer to the SDS Online Help.

   e. Click **Save**.

Perform qPCR on a TaqMan® Array Micro Fluidic Card

For details on running the array, refer to the *TaqMan Array Micro Fluidic Cards User Guide* (PN 4400263).

1. Load the TaqMan Array into a 7900HT Fast instrument that has a TaqMan Array Thermal Cycling Block installed.

   Note: If you are using a 7900HT System with an automation accessory, maximum of 24 TaqMan Arrays can be loaded onto the automation accessory at a time. Arrays containing preamplified product can be kept at room temperature for up to 48 hours (24 arrays × 2 hours/run = 48 hours).

2. Start the run using the default thermal-cycling conditions.

   Note: The SDS software automatically set the appropriate thermal-cycling conditions for the TaqMan Arrays.

   After the run is complete, proceed to "Analyze the experiment" on page 25.

Prepare the preamplification reactions for a TaqMan® Array Plate

Prepare the preamplification reactions (page 22).	Perform thermal cycling (page 18).

When performing the preamplification reactions, see page 8 for product part numbers and sizes.

Recommended amounts of cDNA

For the preamplification reactions, Applied Biosystems recommends that you use:

- 0.2 to 100 ng of cDNA per 30-µL reaction

  Note: Applied Biosystems is developing specific protocols for use with single-cells. For more information, refer to Appendix C on page 35.

- The same amount of cDNA for all reactions

Prepare the preamplification reactions

1. Determine the total number of preamplification reactions to run.

   Note: The 96-well plate formats are laid out in columns. For example, Format 8 has 8 targets in a single column and can accommodate 12 samples as singleton targets.

2. Per the table on page 23, calculate the total volume required for each reaction component:

   volume for 1 reaction × the total number of reactions

Include 20% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Note: If you need to run replicates of the same preamplification products, scale up this reaction volume accordingly. Format 96 or 96+ TaqMan Array Plates contain singleton targets, and an additional plate is required for replicates.

Standard plates are run with 20 µL reaction volume; Fast plates are run with 10 µL reaction. Suggested volumes for TaqMan Array Plates, Standard (Std.) and Fast, are shown:

	TaqMan Array Plate Format				
	Std 8 & Fast 8	**Std 16 & Fast 16**	**Std 32 & Fast 32**	**Std 48 & Fast 48**	**Std 96 & Fast 96**
Number of wells per sample	8	16	32	48	96
PreAmplifcation Reaction Component	**Volume (µL)/Reaction**				
cDNA, 0.2 to 100 ng + nuclease-free water	2	2.5	5	7.5	12.5
Custom TaqMan PreAmp Pool	2	2.5	5	7.5	12.5
TaqMan PreAmp Master Mix (2X)	4	5	10	15	25
Total volume	8	10	20	30	50

3. Thaw the TaqMan PreAmp Master Mix and Custom TaqMan PreAmp Pools on ice. Gently invert the tubes to mix, briefly spin, then return the tubes to ice.
4. In a 0.2-mL reaction tube, tube strip, or 96-well optical reaction plate, combine the required volumes of each reaction component on ice.
5. Cap the reaction tube or seal the reaction plate.
6. Invert the reaction tube or plate several times to mix, then briefly centrifuge.

Perform thermal cycling

Follow the thermal cycling procedure on page 18.

Prepare the PCR amplification reactions for a TaqMan® Array Plate

Prepare the reaction mix (preamplified cDNA + master mix) (this page).	Prepare the TaqMan Array Plate (page 24).	Set up the SDS plate document (page 25).	Run the TaqMan Array Plate on a Real-Time PCR System to perform qPCR (page 25).

Note: When performing the amplification reactions, see page 9 for product part numbers and sizes.

Prepare the reaction mix

Follow steps 1 and 2 in the procedure for preparing the reaction mix on page 19.

Prepare the TaqMan Array Plate

Use a TaqMan Array Plate that contains the TaqMan Gene Expression Assays that target the same genes as your Custom TaqMan PreAmp Pool (with the exception of the 18S gene). For details, refer to the *TaqMan® Arrays Plates Protocol* (PN 4391016).

Suggested volumes for PCR reaction components for TaqMan Array Plates, Standard or Fast, are shown:

	TaqMan Array Plates	Format 8	Format 16 or 16+	Format 32 or 32+	Format 48 or 48+	Format 96 or 96+
	Number of wells per sample	**8**	**16**	**32**	**48**	**96**
	PCR reaction component	**Volume (µL)/Reaction**				
Standard	µL PreAmp Product	4	8	16	24	48
	Nuclease-free water	92	184	368	552	1104
	µL 2X Master Mix	96	192	384	576	1152
	Total volume	192	384	768	1152	2304
Fast	µL PreAmp Product	2	4	8	12	24
	Nuclease-free water	46	92	184	276	552
	µL 2X Master Mix	48	96	192	288	576
	Total volume	96	192	384	576	1152

IMPORTANT! Adding more preamplified cDNA increases the fluorescence baseline and may result in problems with analysis. Volumes for a recommended dilution of 1:48 are shown in the table above.

1. Add the reaction mix to the appropriate wells:
   - Add 20 µL of the reaction mix to each well of a Standard TaqMan Array Plate.

     or
   - Add 10 µL to each well of a Fast TaqMan Array Plate.
2. Seal the TaqMan Array Plate with MicroAmp® optical adhesive film (PN 4360954).
3. Centrifuge the TaqMan Array Plate using a lab centrifuge with plate holder buckets.

Set up the SDS plate document

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

Start the SDS software, 7500 software, or StepOne software.

Download the appropriate text (.txt) file from the Information CD to the real-time PCR system computer.

Refer to the appropriate instrument user guide for:

information on setting up the plate document or experiment,

details on loading and running the plate.

Load the TaqMan Array Plate into a real-time instrument.

Perform qPCR on a TaqMan® Array Plate

1. Load the TaqMan Array Plate into a real-time instrument.

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‡

‡ If you are using a 7900HT System with an automation accessory, then load a maximum of 24 TaqMan Array Plates onto the automation accessory at a time. Plates containing preamplified product can be kept at room temperature for up to 48 hours (24 plates × 2 hours/run = 48 hours).

2. Start the run using the default thermal-cycling conditions.

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.

After the run is complete, proceed to "Analyze the experiment".

Analyze the experiment

The comparative C_T ($\Delta\Delta C_T$) method uses arithmetic formulas to determine the change in expression of a target in an experimental sample relative to the same target in a reference sample. The $\Delta\Delta C_T$ method is used for high-throughput measurements of relative gene expression when there are many genes in many samples. See Appendix B, "Determination of Preamplification Uniformity and Sensitivity," on page 29.

For details on how to analyze $\Delta\Delta C_T$ experiments and set up an RQ Study, refer to the *7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative CT Getting Started Guide* (PN 4364016). Review the chapter on analyzing and viewing RQ Study data in the RQ Manager. Brief procedures for reviewing results and analyzing data are provided below.

Review the data

1. Transfer the SDS plate document file (*.sds) into an RQ Study, then analyze the study. For optimal results, Applied Biosystems recommends:
   - For Applied Biosystems TaqMan master mixes, analyze the study with Automatic Baseline and Manual C_T set to **0.2**.
   - View the amplification plot, then review the baseline and threshold settings. If needed, adjust the baseline and threshold settings.

   IMPORTANT! The same threshold setting must be used for an assay across all samples or arrays within a study.

   Note: You can use either TaqMan Universal PCR Master Mix or TaqMan Gene Expression Master Mix in your experiment. However, Applied Biosystems strongly recommends that you use only one type of master mix per study.

2. In the well table or results table, review the C_T values for each well and for each replicate group. If needed, omit outliers.

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

Perform downstream analysis

For additional analysis, the raw C_T values can be exported as a text file (*.txt), then opened in a spreadsheet application (for example, Microsoft® Excel® Software). If you use SDS software v2.3 or later, you can export the raw C_T values from the Plate Centric view in the RQ Study.

For detailed downstream analysis, Applied Biosystems recommends:

- DataAssist™, a free software package that can be downloaded from:
  www.appliedbiosystems.com/dataassist
- Real-Time StatMiner™ software for detailed statistical analysis. For information, refer to:
  www.integromics.com/StatMiner.php

PCR Good Laboratory Practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- 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. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890).

Determination of Preamplification Uniformity and Sensitivity

Uniformity

In the development of TaqMan® PreAmp Pools, Applied Biosystems has consistently shown that >90% of the genes that are expressed are amplified uniformly without bias. Uniformity of preamplification is estimated based on a comparative C_T ($\Delta\Delta C_T$) method. This method compares the relative quantitation of the unamplified cDNA to the preamplified cDNA from the same sample. A uniformity reference gene is chosen for its consistent gene expression profile. In practice, any gene in the pool can be a reference gene as long as it is not a low- or non-expressing target.

1. For each target, preamplified average C_T (cycle threshold) values are normalized to average C_T values of the uniformity reference gene(s):

   $$\Delta C_{T\ preamp} = \text{avg}\ C_{T\ targetX\ [preamp]} - \text{avg}\ C_{T\ reference\ gene\ [preamp]}$$

2. Likewise, the average C_T values from the cDNA template are normalized to average C_T values from the uniformity reference gene(s):

   $$\Delta C_{T\ cDNA} = \text{avg}\ C_{T\ targetX\ [cDNA]} - \text{avg}\ C_{T\ reference\ gene\ [cDNA]}$$

3. The $\Delta\Delta C_T$ value is determined by the difference between the two ΔC_T values:

   $$\Delta\Delta C_T = \Delta C_{T\ preamp} - \Delta C_{T\ cDNA}$$

On TaqMan Arrays, average C_T values >32 for cDNA samples are often considered to be below the limit of reliable detection. Thus, assays that give C_T values >32 with unamplified cDNA should be omitted from the uniformity calculation. A $\Delta\Delta C_T$ value close to 0 indicates preamplification uniformity. Consistently, >90% of TaqMan Gene Expression Assays produce $\Delta\Delta C_T$ values within ±1.5.

Example of uniformity calculation

The table below shows examples of uniformly amplified targets based on HPRT1 as a reference gene.

Target	UHR cDNA (100 ng per fill reservoir)		× 14 Cycles PreAmp Pools (5 ng of cDNA per reaction, 1:32 dilution)		
	Avg C_T	ΔC_T HPRT1	Avg C_T	ΔC_T HPRT1	$\Delta\Delta C_T$ cDNA-PreAmp Pool
IPO8	26.3	1.8	20.8	2	−0.2
P2RY8	28.1	3.6	22.3	3.5	0.1
PTGER2	30.0	5.5	24.9	6.1	−0.6
PTGER3	29.8	5.3	24.8	6.0	−0.7
HPRT1	24.5	0.0	18.8	0.0	0.0

In this example, 100 ng of unamplified cDNA from Universal Human RNA (UHR) was used per TaqMan Array fill reservoir and compared to 5 ng of the same cDNA sample preamplified with TaqMan PreAmp Pools according to the procedures in "Prepare the preamplification reactions for a TaqMan® Array Micro Fluidic Card" on page 16. The C_T values for the selected targets from unamplified cDNA range from 24 to 30, while the C_T values for the preamplified targets range from 18 to 25. A C_T shift is observed after 14 cycles of preamplification. C_T values are normalized to the HPRT1 gene to calculate the ΔC_T values. Uniformity is indicated by <1.5 ΔC_T difference ($\Delta\Delta C_T$) when comparing preamplification to unamplified cDNA results.

Sensitivity limit

The goal of preamplification is to obtain reliable gene expression data from samples that are available in limited amounts or from samples that have gene targets at low copy number. Ultimately, the goal is to identify differential gene expression between samples.

C_T values using cDNA as sample

In real-time PCR, the C_T value is defined as the number of cycles required for the fluorescence signal to cross the threshold, where the background level is exceeded. A typical quantitative real-time assay has 40 cycles of amplification. C_T values <30 on a TaqMan Array are positive reactions reflecting detectable mRNA target copies in the sample. C_T values between 30 and 32 are positive reactions reflecting low amounts of mRNA targets. C_T values between 32 and 40 reflect very low to null amounts of target.

For low-expressing genes, the confidence in your data improves with:

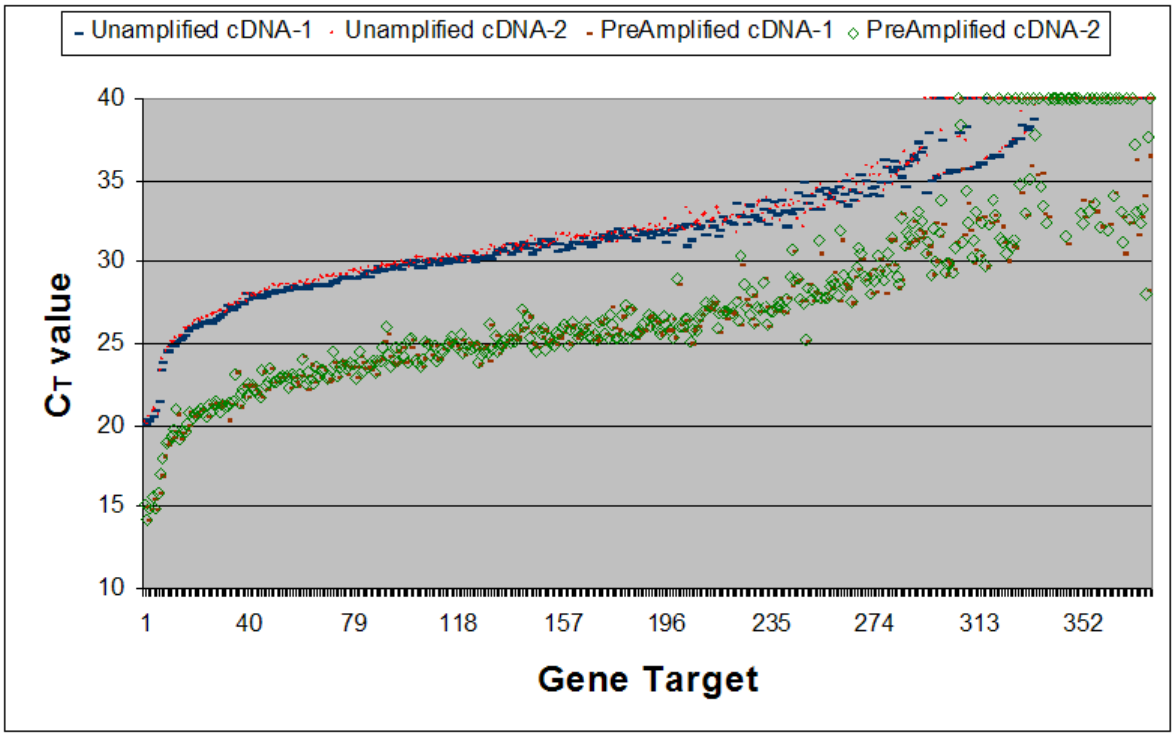
- An increase in the number of replicates run for each sample
- A decrease in the standard deviation between replicates

For a discussion of statistical significance of <2-fold differences in gene expression, refer to the *TaqMan Gene Expression Master Mix Protocol*, Appendix B (PN 4371135).

C_T values using preamplified product as sample

The sensitivity limit for a preamplified product is defined by a C_T cutoff that can be calculated based on cDNA input and the preamplification cycle number. For low-expressing genes, the gene quantitation measurements after preamplification should be below a C_T cutoff. As explained below, C_T values below the cutoff are more reliable than values that are above the cutoff.

The following graph shows the results for unamplified cDNA (100 ng of cDNA per fill reservoir) and preamplified cDNA (5 ng of cDNA per preamplification reaction).



The two technical replicates for both samples show a decrease in variation between replicates with a decrease in the C_T value or with an increase in the target amounts. Also, the ΔC_T shift is constant for the preamplified cDNA relative to the unamplified cDNA at higher copy numbers.

C_T values for unamplified cDNA can be extrapolated to the preamplified cDNA results based on the number of preamplification cycles, dilution, and number of fill reservoirs. If C_T values >32 reflect low to null amounts of target, then the corresponding C_T values for the preamplified cDNA can be extrapolated from the preamplified C_T values. In this example, a C_T value >~26.3 in preamplification corresponds to an unamplified C_T value >32.

Consider the C_T values between 32 and 40 for unamplified cDNA. Targets that fall in this range are considered borderline for positive expression. However, the data could be considered reliable if enough replicates were run and the standard deviation was low. Conversely, the data could be considered unreliable if replicates were not run or if the standard deviation was high.

This also applies to preamplification results. C_T values between 26.3 and 40 should only be considered valid if replicates are reproducible and the standard deviation is low. In this example, C_T values from preamplification of 5 ng of cDNA >C_T 26.3 are outside of the sensitivity limit. Applied Biosystems recommends that you carefully interpret C_T values, or develop an appropriate experimental design, rather than infer expression based on absolute C_T values from preamplification results.

An example of transcript-copy-number-based sensitivity limit

The example in this section provides an alternative way to obtain reliable C_T values from a known input of preamplified product and the preamplification cycle number. All of the C_T values in the preamplification reaction will shift relative to unamplified cDNA, and the shift is constant for a given preamplification input. Moreover, the higher the input, the greater the difference in C_T values for unamplified product compared to preamplified product. The relative shift depends on the input, number of cycles, and copy number. If you calculate a sensitivity limit for cDNA, then it is possible to calculate how much the C_T value will change before running the preamplification reaction.

In this example, an experiment is run using 100 ng of cDNA per fill reservoir as starting template and 5 ng of the same cDNA per preamplification reaction.

Assumptions:

- 1 cell is equivalent to 20 pg of cDNA.
- The cDNA used for preamplification was quantified.
- 100 ng of unamplified cDNA was loaded into 8 fill reservoirs (100 ng per fill reservoir).
- If 1 cell contains 1 copy of a target, then 5000 copies are present in 100 ng (for each fill reservoir).
- 5 ng of starting input for preamplification is equivalent to 250 copies.
- During the first round of preamplification, cDNA is converted from single- to double-stranded product; the number of copies does not increase until the second round.
- The preamplified product was made from 5 ng of cDNA per preamplification reaction using 14 preamplification cycles.

Preamplification Input: 5 ng per Preamplification Reaction			cDNA Input: 100 ng per Fill Reservoir		
Copies	**Preamplification Round**		**Copies**	**LOG_2**	**PCR Round**
250	0		5000	12.3	0
250	1		5000	12.3	1
500	2		10,000	13.3	2
1000	3		20,000	14.3	3
2000	4		40,000	15.3	4
4000	5		80,000	16.3	5
8000	6		160,000	17.3	6
16,000	7		256,000	18.0	6.7
32,000	8		320,000	18.3	7
64,000	9				
128,000	10				
256,000	11				
512,000	12				
1,024,000	13				
2,048,000	14				
256,000	Copies per fill reservoir				

Preamplification input – After 14 rounds of preamplification, under ideal conditions, a total of 2,048,000 copies per preamplification reaction are produced. There are 8 fill reservoirs in a Format 384 Array (for example, the TaqMan Human or Rat GPCR Array). Thus, one-eighth or 256,000 copies were loaded into each fill reservoir starting from 5 ng of cDNA input for preamplification.

cDNA input – Applied Biosystems recommends using 100 ng of cDNA per fill reservoir. Thus, 1 fill reservoir of an unamplified TaqMan Array was loaded with a total of 5000 copies. The increase from 5000 to 256,000 copies takes 5.7 rounds of PCR.

Unamplified RT-PCR started with 5000 copies, and preamplified RT-PCR started with 256,000 copies. Under ideal conditions, the C_T value from preamplified sample will be ~5.7 C_T values lower than the C_T value from unamplified cDNA:

LOG_2 (256,000) –LOG_2 (5000) = 18 –12.3 = 5.7

If C_T values <32 represent positive expression in unamplified cDNA, then:

C_T $threshold_{preamplified\ cDNA}$ = C_T $threshold_{unamplified\ cDNA}$ –$C_{T\ shift}$

32 –5.7 = 26.3

In the example of uniformity shown on page 29, the average C_T values for PTGER2 and PTGER3 are 24.9 and 24.8; these values pass the sensitivity limit of C_T <26.3 for 5 ng of preamplification input. C_T values for cDNA targets also pass the C_T <32 cutoff (that is, 30 and 29.8). Both preamplified and unamplified results are consistent: PTGER2 and PTGER3 targets represent positive, but low-expressing, targets. The calculation for sensitivity limit incorporates cDNA input and the preamplification cycle number. This model provides guidance for evaluating C_T values with preamplification protocols for higher confidence in your results.

Single Cell Gene Expression Analysis Using the TaqMan® Cells-to-CT™ Kit

Kits

Single cell gene expression analysis can be performed on TaqMan® Array Micro Fluidic Cards or TaqMan Array Plates. The following materials are required for single cell gene expression analysis on a Format 96a TaqMan Array Micro Fluidic Card with technical replicates. For other formats of TaqMan Array Cards or for TaqMan Array Plates, volumes may be adjusted at step 4 in the following procedure when adding PreAmp Reagents to the RT Master Mix and diluting the PreAmp product for qPCR:

Product	Part Number
TaqMan Gene Expression Cells-to-CT™ Kit	AM1728 (Ambion)
TaqMan PreAmp Master Mix	4391128 (Applied Biosystems)
TaqMan Gene Expression Master Mix (2X)	4370074 (Applied Biosystems)
Custom TaqMan PreAmp Pools	4441856
TaqMan Custom Array Micro Fluidic Cards:	
Format 12	4342247
Format 16	4346798
Format 24	4342249
Format 32	4346799
Format 48	4342253
Format 64	4346800
Format 96a	4342259
Format 96b	4342261
Format 192	4346802
Format 348	4342265

Procedure

1. Place 1 to 10 cells (~1 µL volume) into 8 µL Cells-to-CT lysis solution. Incubate at room temperature for 5 minutes.

2. Add 1 µl Cells-to-CT stop solution. Incubate at room temperature for 2 minutes. Place samples on ice.

3. Add 15 µL of the RT Master Mix directly to the 10 µL Cells-to-CT lysate to make a 25 µL RT reaction. Determine the number of samples and use the following chart to make the RT Master Mix:

Component	25 µL RT reaction per single cell
2X RT Buffer	12.5 µL
20X RT Enzyme Mix	1.25 µL
Nuclease-free water	1.25 µL
Final Volume RT Master Mix	15 µL

Incubate at 37 °C for 60 minutes, then at 95 °C for 5 minutes to inactivate the RT enzyme.

4. Add PreAmp reagents to the RT Master Mix:

2X PreAmp Master Mix	50 µL
0.2X Pooled Assay Mix	25 µL

Place the preamplification tubes in a thermal cycler and run using these cycling conditions:

Hold	Cycle (14 cycles)		Hold	Hold
	Denature	Anneal/Extend		
95 °C	95 °C	60 °C	99.9 °C	4 °C
10 min	15 sec	4 min	10 min	∞

Place the tubes on ice or store at 4 °C. Alternatively, you can store the preamplified product at –20 °C for up to 2 weeks.

Dilute the PreAmp products 1:64 with 0.1X TE pH 8.0 before using them in a Real-Time PCR instrument.

Set up the real-time PCR TaqMan Micro Fluidic Array Card using these reagent amounts:

Component	440 µL/4 fill ports per sample
TaqMan Gene Expression Master Mix (2X)	220 µL
Diluted PreAmp product	220 µL
Final volume	440 µL

Note: When 4 ports of a Format 96a card are loaded, there are 2 technical replicates.

5. Record the C_T values for each well:
   a. View the amplification plots for the entire plate and check for any outlier.
   b. Set the baseline and threshold values (automatic baseline and threshold of 0.2 is recommended).
   c. Analyze and export the data file (.txt).
   d. Set up a C_T cutoff. Refer to Appendix B, "Determination of Preamplification Uniformity and Sensitivity" on page 29 to determine the appropriate cutoff.
   e. Perform data analysis using DataAssist™, StatMiner™ or other qPCR data analysis software.

Perform the run

Refer to the procedures "Perform qPCR on a TaqMan® Array Micro Fluidic Card" on page 22 and "Perform qPCR on a TaqMan® Array Plate" on page 25.

Analyze the experiment

Analyze the experiment according to the procedure on page 25.

Safety

This appendix covers:

Chemical safety

General chemical safety

Chemical hazard warning

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

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

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

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

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 41.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

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

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

Obtaining SDSs

The SDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. To obtain SDSs:

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

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

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

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

Waste disposal

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

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

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

Biological hazard safety

General biohazard

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

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (**www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**).
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

General safety alerts for all chemicals

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

CAUTION! CHEMICAL HAZARD. TaqMan® Universal PCR Master Mix may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION! CHEMICAL HAZARD. TaqMan Gene Expression Master Mix is harmful if swallowed. Causes eye, skin, and respiratory tract irritation. Read Safety Data Sheet and follow the handling instructions. Wear protective eyewear, clothing, and gloves and use with adequate ventilation.

Documentation and Support

Related documentation

The following related documents are shipped with the system:

Document	Part number
Applied Biosystems 9800 Fast Thermal Cycler User Guide	4350087
Applied Biosystems Real-Time PCR Systems Reagent Guide	4387787
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 CT Getting Started Guide	4364016
GeneAmp® PCR System 9700 Base Module: User's Manual	4303481
High Capacity RNA-to-cDNA Kit Protocol	4387951
High-Capacity cDNA Reverse Transcription Kits Protocol	4375575
RecoverAll™ Total Nucleic Acid Isolation Kit Protocol	1975M‡
RNAqueous®-Micro Kit Protocol	1911M§
TaqMan® Array Micro Fluidic Cards User Guide	4400263
TaqMan Array Plates Protocol	4391016
TaqMan Gene Expression Cells-to-CT™ Kit Protocol	4385117
Custom TaqMan Gene Expression Master Mix Protocol	4371135
TaqMan PreAmp Master Mix Kit Protocol	4384557
TaqMan Universal PCR Master Mix Protocol	4304449
TaqMan Universal Master Mix II Protocol	4428173
TaqMan Universal Master Mix II Quick Reference Card	4428174

‡ The RecoverAll Total Nucleic Acid Isolation Kit Protocol (PN 0511) can be downloaded from **www.ambion.com**.

§ The *RNAqueous-Micro Kit Protocol* (PN 0410) can be downloaded from **www.ambion.com**.

Note: For additional documentation, see "Obtaining support" on page 46.

Obtaining support

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

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

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

Index

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www.appliedbiosystems.com/support