applied biosystems

TaqMan® Gene Expression Master Mix

USER GUIDE

For two-step RT-PCR in gene expression experiments or quantitative analysis

Catalog Numbers 4369016, 4369510, 4369514, 4369542, 4370048, and 4370074

Publication Number 4371135

Revision D

For Research Use Only. Not for use in diagnostic procedures.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 4371135

Revision	Date	Description
D	11 February 2019	• Added new instruments and other applicable products. • Updated procedural guidelines. • Added procedures for TaqMan® Array Plates and TaqMan® Array Cards. • Updated troubleshooting section. • Updated for general style, formatting and branding.
C	July 2010	Baseline for this revision history.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license. Cy3 and Cy5 are trademarks of GE Healthcare.

©2019 Thermo Fisher Scientific Inc. All rights reserved.

Contents

- **CHAPTER 1** Product information 6
  - Product description 6
  - Contents and storage 7
  - Required materials and equipment not supplied 7
  - Workflow 9
- **CHAPTER 2** RT-PCR for TaqMan® and Custom TaqMan® Gene Expression Assays—single-tube assays 10
  - Prepare cDNA 10
    - Guidelines for isolation of high-quality RNA 10
    - Guidelines for preparing cDNA templates 10
    - Perform reverse transcription 10
  - Perform PCR 11
    - Guidelines for PCR 11
    - Before you begin 11
    - Prepare PCR reaction mix 12
    - Prepare the PCR reaction plate 12
    - Set up a plate file 13
    - Run the PCR reaction plate 13
  - Analyze data 14
    - Algorithms for data analysis 14
- **CHAPTER 3** RT-PCR for TaqMan® Gene Expression Assays—TaqMan® Array Plates 15
  - Prepare cDNA 15
    - Guidelines for isolation of high-quality RNA 15
    - Guidelines for preparing cDNA templates 15
    - Perform reverse transcription 15
  - Perform PCR 16
    - Guidelines for PCR 16
    - Before you begin 16
    - Prepare the PCR reaction mix 16
    - Prepare the PCR reaction plate 16

Set up a plate document or experiment file ... 17
Run the PCR reaction plate ... 17
Analyze data ... 18
Algorithms for data analysis ... 18

CHAPTER 4 RT–PCR for TaqMan® Gene Expression Assays—TaqMan® Array Cards ... 19

Prepare cDNA ... 19
Guidelines for isolation of high–quality RNA ... 19
Guidelines for preparing cDNA templates ... 19
Perform reverse transcription ... 19
Perform PCR ... 20
Guidelines for PCR ... 20
Recommended amount of cDNA ... 20
Before you begin ... 20
Prepare the PCR reaction mix ... 21
Prepare the TaqMan® Array Card ... 21
Set up a card file ... 21
Run the TaqMan® Array Card ... 22
Analyze data ... 23
Algorithms for data analysis ... 23

APPENDIX A Troubleshooting ... 24

APPENDIX B Supplemental information ... 28

Components of the TaqMan® Gene Expression Master Mix ... 28
AmpliTaq Gold™ DNA Polymerase, UP (Ultra Pure) ... 28
Uracil-N glycosylase ... 28
ROX™ Passive Reference dye ... 28
Two–step RT–PCR ... 28
Overview of TaqMan® Gene Expression Assays ... 30
TaqMan® MGB probes ... 30
About the 5' nuclease assay ... 30
Dyes for multicomponent analysis ... 31
How to calculate R_n and ΔR_n Values ... 32
Guidelines for duplex reactions using TaqMan® Gene Expression Assays ... 32
Best practices for PCR and RT-PCR experiments ... 33
Good laboratory practices for PCR and RT-PCR ... 33
Use UNG to prevent false-positive amplification ... 33
Detect fluorescent contaminants ... 33

APPENDIX C Safety 34

Chemical safety 35
Biological hazard safety 36

Documentation and support 37

Related documentation 37
Customer and technical support 38
Limited product warranty 39

Product information

- Product description 6
- Contents and storage 7
- Required materials and equipment not supplied 7
- Workflow 9

Product description

The Applied Biosystems™ TaqMan® Gene Expression Master Mix can be used with any of the following real-time PCR applications.

- Pathogen detection
- Copy number analysis (gene dosage analysis)
- Microarray validation
- Differential gene expression analysis
- Viral load quantification
- Methylation analysis
- MicroRNA quantification

Note: The TaqMan® Gene Expression Master Mix is not compatible with TaqMan® Advanced miRNA Assays.

Use the Master Mix with complimentary DNA (cDNA), genomic DNA (gDNA), or plasmid DNA. The Master Mix is supplied at a 2X concentration and contains the following components:

- AmpliTaq Gold™ DNA Polymerase, UP (Ultra Pure)
- Uracil-DNA glycosylase
- dNTP with dUTP
- ROX™ dye (passive reference)
- Optimized buffer components

For more information about each component, see "Components of the TaqMan® Gene Expression Master Mix" on page 28.

Contents and storage

Table 1 TaqMan® Gene Expression Master Mix

Cat. No.	Number of 50-µL reactions	Amount	Storage[1]
4370048	40	1 × 1 mL	2–8°C
4369016	200	1 × 5 mL	
4369514	400	2 × 5 mL	
4369510	1000	5 × 5 mL	
4369542	2000	10 × 5 mL	
4370074	2000	1 × 50 mL	

[1] See label for expiration date.

Required materials and equipment not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instrument, one of the following:	
QuantStudio™ 3 and 5 Real-Time PCR Instruments[1]	Contact your local sales office
QuantStudio™ 6 Flex Real-Time PCR System[1]	
QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
StepOne™ Real-Time PCR System[2]	
StepOnePlus™ Real-Time PCR System	
7500 Real-Time PCR System[1]	
7500 Fast Real-Time PCR System[1]	
7900HT Fast Real-Time PCR Instrument	
7900HT Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
Equipment	
Centrifuge with plate adapter	MLS
Microcentrifuge	

Item	Source
Laboratory mixer (vortex or equivalent)	MLS
Pipettes	
Tubes, plates and other consumables	
Plastic consumables	thermofisher.com/plastics
Pipette tips	thermofisher.com/pipettetips
Disposable gloves	MLS

[1] Not compatible with TaqMan® Array Cards.
[2] Not compatible with TaqMan® Array Cards or TaqMan® Array Plates.

Table 2 Kits and reagents for RNA isolation

Item	Source
RNA isolation products	thermofisher.com/rnaisolation
Supporting reagents	thermofisher.com/rnaisolationreagents

Table 3 Reagents for reverse transcription

Item	Source
Reagents for reverse transcription (all assays)	
TE, pH 8.0	AM9849
(Optional) RNase Inhibitor	N8080119 AM2682
RNase-free, sterile-filtered water	MLS
Reverse transcription kit, one of the following:	
TaqMan® Reverse Transcription Reagents	N8080234
High-Capacity cDNA Reverse Transcription Kit	4368814 4374966 (with RNase inhibitor)
High-Capacity RNA-to-cDNA™ Kit	4387406
SuperScript™ IV VILO™ Master Mix	11756050

Table 4 Assays

Item	Source
TaqMan® Assays	
TaqMan® Gene Expression Assays	thermofisher.com/taqmangeneexpression
Custom TaqMan® Gene Expression Assays	thermofisher.com/taqmancustomgeneexpression
Custom TaqMan® probes and primers[1]	thermofisher.com/customprimersprobes
TaqMan® Array Plates	
TaqMan® 96-well Standard (0.2-mL) Plates and 96-well Fast (0.1-mL) Plates	thermofisher.com/taqmanarrays
TaqMan® Array Cards	
TaqMan® Array Cards	thermofisher.com/taqmanarrays

[1] Synthesized to your sequence and choice of quencher and reporter dyes.

Workflow

Perform reverse transcription

Perform real-time PCR amplification

Prepare the PCR reaction mix

Prepare the PCR reaction plate, TaqMan® Array Plates, or TaqMan® Array Cards

Set up a plate or card file

(or use the document provided with the cards or custom plates)

Run the PCR reaction plate

Analyze the data

RT–PCR for TaqMan® and Custom TaqMan® Gene Expression Assays—single–tube assays

Prepare cDNA

Guidelines for isolation of high–quality RNA

- For recommended RNA isolation kits, see Table 2 on page 8.
- *(Optional)* Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparing cDNA templates

- Use the same reverse transcription procedure for all samples.
- For optimal reverse transcription, input RNA should be:
  - Free of inhibitors of reverse transcription (RT) and PCR
  - Dissolved in PCR-compatible buffer
  - Free of RNase activity

    Note: We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA (not applicable for double-stranded templates)

    IMPORTANT! Degradation of the RNA may reduce the yield of cDNA for some gene targets.
- For the input RNA amount, follow the recommendations provided by the cDNA kit.
- Small amounts of cDNA can be pre-amplified.
  Use TaqMan® PreAmp Master Mix (Cat. No. 4391128) or TaqMan® PreAmp Master Mix Kit (Cat. No. 4384267).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.
- If using strip tubes to prepare cDNA templates, change to a new cap after each step or incubation.
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes to prepare cDNA templates.

Perform reverse transcription

Perform reverse transcription to obtain cDNA from RNA samples.

For information on reverse transcription kits, see Table 3 on page 8. For detailed guidelines and instructions, see *TaqMan® Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458).

Perform PCR

Guidelines for PCR

- Store the TaqMan® Assays frozen.
- Protect from light until use. Excessive exposure to light might affect the fluorescent probes.
- Multiple assays can be run on one reaction plate. Include no-template controls (NTCs) for each assay.
- For duplex reactions, run reactions in singleplex before duplex to ensure that the addition of a second assay does not inhibit reactions. For more information, see “Guidelines for duplex reactions using TaqMan® Gene Expression Assays“ on page 32.

Before you begin

Dilute 60X assays to 20X working stocks with TE, pH 8.0. Divide the solutions into smaller aliquots to minimize freeze–thaw cycles. The aliquot size depends on how many PCR reactions you will run.

- Determine the total number of PCR reactions needed, including replicates, for each sample. Include a no template control for each assay.

  Note: We recommend four replicates for each assay.

- Mix the Master Mix thoroughly but gently.
- Thaw the TaqMan® Assays on ice, then vortex and briefly centrifuge to resuspend.
- Thaw samples on ice, then vortex and briefly centrifuge to resuspend.

Prepare PCR reaction mix

1. Combine the following components in the quantities shown, multiplied by the number of reactions required. Add 10% overage for pipetting loss.

<table>
<tr><th rowspan="3">Component</th><th colspan="4">Volume per reaction</th><th rowspan="3">Final concentration</th></tr>
<tr><th colspan="2">384-well plate, 96- or 48-well fast plate</th><th colspan="2">Standard 96-well plate</th></tr>
<tr><th>Singleplex</th><th>Duplex</th><th>Singleplex</th><th>Duplex</th></tr>
<tr><td>TaqMan® Gene Expression Master Mix (2X)</td><td>5.0 µL</td><td>5.0 µL</td><td>10.0 µL</td><td>10.0 µL</td><td>1X</td></tr>
<tr><td>TaqMan® Assay (20X)</td><td>0.5 µL</td><td>0.5 µL</td><td>1.0 µL</td><td>1.0 µL</td><td>1X</td></tr>
<tr><td>TaqMan® endogenous control (20X)</td><td>—</td><td>0.5 µL</td><td>—</td><td>1.0 µL</td><td>—</td></tr>
<tr><td>cDNA template + Nuclease-free Water[1]</td><td>4.5 µL</td><td>4.0 µL</td><td>9.0 µL</td><td>8.0 µL</td><td>—</td></tr>
<tr><td>Total PCR Reaction Mix volume</td><td>10 µL</td><td>10 µL</td><td>20 µL</td><td>20 µL</td><td>—</td></tr>
</table>

[1] Use 10 to 100 ng of cDNA. Adjust the volume of Nuclease-free Water in the PCR reaction mix for a larger volume of cDNA.

2. Vortex briefly to mix.
3. Centrifuge briefly to collect the contents at the bottom of the tube.

Prepare the PCR reaction plate

1. Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.
   - 384-well plate, 96-well fast plate, 48-well plate: 10 µL
   - Standard 96-well plate: 20 µL
2. Seal the reaction plate with optical adhesive film.
3. Centrifuge the plate briefly to collect the contents at the bottom of the plate.
4. Apply a compression pad to the plate, if required by your real-time PCR system.

Set up a plate file

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

Note: The instrument must be configured with the block appropriate for the plate type.

1. Set up the thermal protocol.

UNG incubation[1]	Polymerase activation [2]	PCR (40 cycles)	
Hold 50°C	Hold 95°C	Denature 95°C	Anneal / extend 60°C
2 minutes	10 minutes	15 seconds	60 seconds

[1] For optimal UNG activity.
[2] To activate AmpliTaq Gold™ DNA Polymerase, UP.

2. Select the appropriate block, if this option applies to your instrument.
3. Select the appropriate experiment type, if this option applies to your instrument.
4. Select **TaqMan® Reagents** to detect the target sequence, if this option applies to your instrument.
5. Select the standard cycling mode.

   IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on the Standard or Fast plate format.

6. Assign targets and samples.
7. Enter the sample volume, if it applies to your instrument.
   - 384-well plate, 96-well Fast plate, 48-well plate: 10 µL
   - Standard 96-well plate: 20 µL

Run the PCR reaction plate

1. Open the plate file that corresponds to the reaction plate in the system.
2. Load the reaction plate.
3. Start the run.

Analyze data

Data analysis varies depending on your real–time PCR system. See the instrument user guide for more information.

1. View the amplification plots for the reactions.
2. Use auto baseline and auto threshold settings, or set the baseline and threshold values to determine the threshold cycles (C_t) for the amplification curves.
3. Use the relative standard curve method or the comparative C_t method to analyze data.

Algorithms for data analysis

Table 5 Algorithm recommendations for single–tube assays

Algorithm	Recommendation
Threshold (C_t)	Recommended.
Relative threshold (C_{rt})	*(Optional)* Use for troubleshooting abnormal or unexpected results.

The relative threshold algorithm is available in the Relative Quantification application on the Thermo Fisher Cloud (**thermofisher.com/cloud**).

RT–PCR for TaqMan® Gene Expression Assays—TaqMan® Array Plates

Prepare cDNA

Guidelines for isolation of high–quality RNA

- For recommended RNA isolation kits, see Table 2 on page 8.
- *(Optional)* Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparing cDNA templates

- Use the same reverse transcription procedure for all samples.
- For optimal reverse transcription, input RNA should be:
  - Free of inhibitors of reverse transcription (RT) and PCR
  - Dissolved in PCR-compatible buffer
  - Free of RNase activity

    Note: We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA (not applicable for double-stranded templates)

    IMPORTANT! Degradation of the RNA may reduce the yield of cDNA for some gene targets.
- For the input RNA amount, follow the recommendations provided by the cDNA kit.
- Small amounts of cDNA can be pre-amplified.
  Use TaqMan® PreAmp Master Mix (Cat. No. 4391128) or TaqMan® PreAmp Master Mix Kit (Cat. No. 4384267).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.
- If using strip tubes to prepare cDNA templates, change to a new cap after each step or incubation.
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes to prepare cDNA templates.

Perform reverse transcription

Perform reverse transcription to obtain cDNA from RNA samples.

For information on reverse transcription kits, see Table 3. For detailed guidelines and instructions, see *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Plates* (Pub. No. 4391016).

Perform PCR

Guidelines for PCR

Store TaqMan® Array Plates away from light until use. Excessive exposure to light may affect the fluorescent probes.

Before you begin

- Determine the total number of PCR reactions. One reaction corresponds to one well in the plate.
- Mix the Master Mix thoroughly but gently.
- Thaw samples on ice, then vortex and briefly centrifuge to resuspend.

Prepare the PCR reaction mix

1. Combine the following components for the number of reactions required. Add 10% overage for pipetting loss.

Component	Volume per reaction	
	96-well Fast (0.1-mL) Plate	96-well Standard (0.2-mL) Plate
cDNA template + Nuclease-free Water[1]	5 µL[2]	10 µL[3]
TaqMan® Gene Expression Master Mix (2X)	5 µL	10 µL
PCR Reaction Mix volume	10 µL	20 µL

[1] Adjust the volume of Nuclease-free Water in the PCR reaction mix for a larger volume of cDNA
[2] Ensure that the final cDNA concentration per well is 5-50 ng per reaction.
[3] Ensure that the final cDNA concentration per well is 1-100 ng per reaction.

2. Vortex briefly to mix.
3. Centrifuge briefly to bring the reaction mix to the bottom of the tube.

Prepare the PCR reaction plate

1. Transfer the appropriate volume of PCR Reaction Mix to each well of the plate.
   - 96–well fast (0.1 mL) plate: 10 µL
   - 96–well standard (0.2 mL) plate: 20 µL
2. Seal the plate with optical adhesive film.
3. Centrifuge the plate briefly to collect the contents at the bottom of the plate.
4. Apply a compression pad to the plate, if required by your real-time PCR system.

Set up a plate document or experiment file

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

Download the setup file at **thermofisher.com/taqmanfiles**.

Note: The instrument must be configured with the block appropriate for the plate type.

1. Import the setup file (SDS in TXT format) into the real-time PCR instrument software.
2. Set up the thermal protocol.

UNG incubation[1]	Polymerase activation [2]	PCR (40 cycles)	
Hold 50°C	Hold 95°C	Denature 95°C	Anneal / extend 60°C
2 minutes	10 minutes	15 seconds	60 seconds

[1] For optimal UNG activity.
[2] To activate AmpliTaq Gold™ DNA Polymerase, UP.

3. Select the appropriate block, if this option applies to your instrument.
4. Select the appropriate experiment type, if this option applies to your instrument.
5. Select **TaqMan® Reagents** to detect the target sequence, if this option applies to your instrument.
6. Select the standard cycling mode.

IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on the Standard or Fast plate format.

7. Enter the sample volume, if it applies to your instrument.
   - 96-well fast (0.1 mL) plate: 10 µL
   - 96-well standard (0.2 mL) plate: 20 µL

Run the PCR reaction plate

1. Open the plate file that corresponds to the reaction plate in the system.
2. Load the reaction plate.
3. Start the run.

Analyze data

Data analysis varies depending on your real–time PCR system. See the instrument user guide for more information.

1. View the amplification plots for the reactions.
2. Set the baseline and threshold values to determine the threshold cycles (C_t) for the amplification curves, or select relative threshold under analysis settings to obtain (C_{rt}) values.
3. Use the relative standard curve method or the comparative C_t method to analyze data.

Algorithms for data analysis

Table 6 Algorithm recommendations for TaqMan® Array Plates

Algorithm	Recommendation
Threshold (C_t)	• Recommended for data analysis.
Relative threshold (C_{rt})	• *(Optional)* Use for data analysis. • Use to troubleshoot unexpected results. • Use to correct a variable baseline, which can be due to dried-down assays on the plate being reconstituted at different rates.

The relative threshold algorithm is available in the Relative Quantification application on the Thermo Fisher Cloud (**thermofisher.com/cloud**).

RT–PCR for TaqMan® Gene Expression Assays— TaqMan® Array Cards

Prepare cDNA

Guidelines for isolation of high–quality RNA

- For recommended RNA isolation kits, see Table 2 on page 8.
- *(Optional)* Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparing cDNA templates

- Use the same reverse transcription procedure for all samples.
- For optimal reverse transcription, input RNA should be:
  - Free of inhibitors of reverse transcription (RT) and PCR
  - Dissolved in PCR-compatible buffer
  - Free of RNase activity

    Note: We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA (not applicable for double-stranded templates)

    IMPORTANT! Degradation of the RNA may reduce the yield of cDNA for some gene targets.
- For the input RNA amount, follow the recommendations provided by the cDNA kit.
- Small amounts of cDNA can be pre-amplified.
  Use TaqMan® PreAmp Master Mix (Cat. No. 4391128) or TaqMan® PreAmp Master Mix Kit (Cat. No. 4384267).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.
- If using strip tubes to prepare cDNA templates, change to a new cap after each step or incubation.
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes to prepare cDNA templates.

Perform reverse transcription

Perform reverse transcription to obtain cDNA from RNA samples.

For reverse transcription kits, see Table 3 on page 8. For detailed instructions, see *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Cards* (Pub. No. 4400263).

Perform PCR

Guidelines for PCR

- Store the TaqMan® Array Card in its packaging until the packaging has reached room temperature and you are ready to fill it with sample–specific PCR mix.
- Protect from light. Prolonged exposure to indoor lighting can degrade the fluorescent probes in the card. Do not expose the card to sunlight.
- Fill each fill reservoir with sample–specific PCR mix made from a single cDNA sample.
- Use 100 µL of sample–specific PCR mix to fill each fill reservoir. Volumes smaller than 100 µL will result in insufficiently filled cards.
- Do not add the sample after centrifuging the cards. Centrifugation of the card causes the sample–specific PCR mix to resuspend the dried TaqMan® probes and primers within the wells of the card. Addition of the sample after centrifuging disrupts the resuspended assay positions.
- After loading the card with PCR reaction mix, equilibrate the card to room temperature before loading it into the real–time PCR instrument.
- Run the card within 72 hours of sealing it.
- Protect the card from light and store at 2–8°C if a run is not started immediately after sealing.

Recommended amount of cDNA

- We recommend 30–1000 ng (0.3–10 ng/µL) of cDNA (converted from total RNA) per fill reservoir.
- The amount of cDNA to use depends on the expression level of the target genes and the number of target copies per well that need to be detected. For example:
  - Use 1000 ng (10 ng/µL) per fill reservoir to detect genes with low expression. Because the cDNA concentration is high, use high-quality cDNA without inhibitors.
  - Use 100–200 ng per fill reservoir to detect genes with moderate expression.
  - Use 30–50 ng per fill reservoir to detect genes with moderate to high expression.
- Use the same amount of cDNA sample for all reactions.

Before you begin

- Determine the number of fill reservoirs in the array card that will be used for each cDNA sample.
- Thaw samples on ice, then vortex and briefly centrifuge to resuspend.
- Mix the Master Mix thoroughly but gently.

Prepare the PCR reaction mix

1. Combine the following components for the number of reactions required. Add 10% overage for pipetting loss.

Component	Volume per fill reservoir
cDNA template + Nuclease-free Water[1]	50 µL
TaqMan® Gene Expression Master Mix (2X)	50 µL
PCR Reaction Mix volume	**100 µL**

[1] See "Recommended amount of cDNA" on page 20.

2. Vortex briefly to mix.

3. Centrifuge briefly to bring the reaction mix to the bottom of the tube.

Prepare the TaqMan® Array Card

Fill the TaqMan® Array Card with sample–specific PCR Reaction Mix, then centrifuge and seal the card.

For detailed procedures to prepare the card, see *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Cards* (Pub. No. 4400263).

Set up a card file

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

Download the setup file at **thermofisher.com/taqmanfiles**.

Note: The instrument must be configured with the block appropriate for the plate type.

1. Import the setup file (SDS in TXT format) into the real-time PCR instrument or software.

2. Set up the thermal protocol.

   Note: Thermal cycling conditions depend on the instrument.

Table 7 Compatible QuantStudio™ systems and ViiA™ 7 Real-Time PCR Systems

UNG incubation[1]	Polymerase activation[2]	PCR (40 cycles)	
Hold 50°C	Hold 95°C	Denature 95°C	Anneal / extend 60°C
2 minutes	10 minutes[3]	15 seconds	60 seconds

[1] For optimal UNG activity.
[2] To activate AmpliTaq Gold™ DNA Polymerase, UP.
[3] To completely dissolve the primers and probes on the TaqMan® Array Card .

Table 8 7900HT Fast Real-Time PCR Instrument

UNG incubation[1]	Polymerase activation[2]	PCR (40 cycles)	
Hold 50°C	**Hold 94.5°C**	**Denature 97°C**	**Anneal / extend 59.7°C**
2 minutes	10 minutes[3]	30 seconds	60 seconds

[1] For optimal UNG activity.
[2] To activate AmpliTaq Gold™ DNA Polymerase, UP.
[3] To completely dissolve the primers and probes on the TaqMan® Array Card.

3. Select the appropriate block, if this option applies to your instrument.
4. Select the appropriate experiment type, if this option applies to your instrument.
5. Select **TaqMan® Reagents** to detect the target sequence, if this option applies to your instrument.
6. Select the standard cycling mode.

   IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction.

7. Confirm that the sample volume is set to 1.0 µL.

Run the TaqMan® Array Card

1. Open the card file that corresponds to the array card in the system.
2. Load the array card.
3. Start run.

Analyze data

Data analysis varies depending on your real–time PCR system. See the instrument user guide for more information.

1. View the amplification plots for the reactions.
2. Use auto baseline and auto threshold settings, or set the baseline and threshold values to determine the threshold cycles (C_t) for the amplification curves.
3. Use the relative standard curve method or the comparative C_t method to analyze data.

Algorithms for data analysis

Table 9 Algorithm recommendations for TaqMan® Array Cards

Algorithm	Recommendation
Relative threshold (C_{rt})	Recommended for the following instruments: • QuantStudio™ Real-Time PCR Instruments • ViiA™ 7 instrument Can correct a variable baseline, which might be due to dried-down assays on the card being reconstituted at different rates.
Threshold (C_t)	Optional if used for analysis of established protocols. Recommended for 7900HT Fast Real-Time PCR Instrument.

The relative threshold algorithm is available in the Relative Quantification application on the Thermo Fisher Cloud (**thermofisher.com/cloud**).

Troubleshooting

Observation	Possible cause	Recommended action
The ΔR_n is less than or equal to No Template Control ΔR_n, and there is no amplification plot	Inappropriate reaction conditions were used.	Troubleshoot RT optimization and the PCR optimization.
	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.
	One or more of the reaction components were not added.	Check your pipetting equipment and technique.
	(Custom TaqMan® Gene Expression Assays only) Incorrect primer or probe sequence.	Reorder the assay with the appropriate sequence.
	The template is degraded or was not added.	• Determine the quality of the template. • Rerun the assay with fresh template. • Use RNase-free reagents. • Use an RNase inhibitor.
	Inhibitors are present in the reaction.	Ensure the presence of an inhibitor: 1. Create a serial dilution of your sample. 2. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, high concentrations yield higher-then-expected Ct values. (High concentration means more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template.
The ΔR_n is less than or equal to No Template Control ΔR_n, and both reactions show an amplification plot	The reagents are contaminated with gDNA, amplicon, or plasmid clones.	• Rerun the assay using reagents. • Ensure that your workspace and equipment are cleaned properly. • Run no-RT controls to rule out genomic DNA contamination. • Treat the sample with DNase. • Design an assay that spans an exon-exon boundary if genomic DNA contamination is suspected.

Observation	Possible cause	Recommended action
There was a shifting Rn value during the early cycles of the PCR (cycles 0 to 5)	Fluorescence did not stabilize to the buffer conditions of the reaction mix. **Note:** This condition does not affect PCR or the final results.	• Reset the lower value of the baseline range. • Use an automatic baseline. • Use the relative threshold algorithm (C_{rt}). See *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239).
Amplification curve shows abnormal plot and/or low ΔRn values Linear view: Log view:	The baseline was set improperly (some samples have C_t values lower than the baseline stop value).	See your real-time PCR system user guide for procedures on setting the baseline. Switch from manual to automatic baselining, or move the baseline stop value to a lower C_t (2 cycles before the amplification curve crosses the threshold). Corrected log view:
	An amplification signal is detected in the early cycles (no baseline can be set because the signal is detected too early).	Dilute the sample to increase the C_t value.
The multicomponent signal for ROX™ dye is not flat	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.
There was a small ΔRn	The PCR efficiency was poor.	Ensure that the reagents were used at the correct concentration.
	The quantity of the cDNA is low (a low copy number of the target).	Increase the quantity of the cDNA.
Lower ΔRn values were obtained in early cycles	The C_t value is less than 15.	Adjust the upper baseline range to a value less than 15.

<table>
<tr><th>Observation</th><th>Possible cause</th><th>Recommended action</th></tr>
<tr><td rowspan="2">The ΔR_n or R_n values are high</td><td>The ROX™ dye was not set as the passive reference.</td><td>Set ROX™ dye as the passive reference, then reanalyze the data.</td></tr>
<tr><td>The sample evaporated.</td><td>Check the seal of the adhesive film for leaks.</td></tr>
<tr><td rowspan="4">There is a high standard deviation in the replicates, inconsistent data, or a variable C_t</td><td>The reagents were not mixed properly.</td><td>• Increase the length of time that you mix the reagents.
• Verify your mixing process by running a replicate assay.</td></tr>
<tr><td>Pipetting was inaccurate.</td><td>• Check the calibration of the pipettes.
• Pipette at least 5 µL of sample to prepare the reaction mix.</td></tr>
<tr><td>The threshold was not set correctly.</td><td>Set the threshold above the noise level and where the replicates are tightest. See your real-time PCR system user documentation.</td></tr>
<tr><td>There was a low concentration of the target of interest.</td><td>Rerun the assay using more cDNA template.</td></tr>
<tr><td rowspan="2">Amplification curve shows no amplification of the sample (C_t=40) in the target assay</td><td>The gene is not expressed in the tested sample.</td><td>• Ensure that the gene is expressed in the sample type or tissue type.
Go to ncbi.nlm.nih.gov/unigene.
• Confirm the results.
– Rerun the sample using the same assay.
– Rerun the experiment using more sample. Avoid preparing PCR reaction mixes with more than 20% reverse transcription reaction.
– Run the experiment using an alternative assay, if available, that detects a different transcript or more than one transcript from the same gene.

Note: If the recommended actions do not resolve the problem, the result may be correct.</td></tr>
<tr><td>The sample does not have enough copies of the target RNA.</td><td>Confirm the results.
• Rerun the sample using the same assay.
• Rerun the assay using more sample. Avoid PCR reaction mix with more than 20% from the reverse transcription reaction.

Note: If the recommended actions do not resolve the problem, the result may be correct.</td></tr>
</table>

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (C_t=40) in the target assay	One or more of the reaction components was not added.	Check your pipetting equipment and/or technique.
	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.

Supplemental information

Components of the TaqMan® Gene Expression Master Mix

AmpliTaq Gold™ DNA Polymerase, UP (Ultra Pure)

AmpliTaq Gold™ DNA Polymerase, UP (Ultra Pure) is purified through a propriety process to reduce the bacterial DNA introduced from the host organism. The purification process reduces the amount of non-specific and false-positive DNA products generated during PCR from bacterial DNA contamination.

When AmpliTaq Gold™ DNA Polymerase, UP is added to the reaction mixture at room temperature, the inactive enzyme is not capable of primer extension. Any low-stringency mispriming events that may occur will not be enzymatically extended and subsequently amplified. A thermal incubation step is required for activation to ensure that active enzyme is generated only at temperatures where the DNA is fully denatured.

Uracil-N glycosylase

Uracil-N glycosylase (UNG) treatment can prevent the reamplification of carryover PCR products by removing any uracil incorporated into single- or double-stranded amplicons. UNG prevents reamplification of carryover PCR products in an assay if all previous PCR for that assay was performed using a dUTP-containing master mix. See "Use UNG to prevent false-positive amplification" on page 33 for more information about UNG.

ROX™ Passive Reference dye

The ROX™ Passive Reference dye provides an internal reference to which the reporter dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescent fluctuations due to changes in concentration or volume.

Two–step RT–PCR

Visit **thermofisher.com/qpcreducation** for more information.

A target template is a DNA sequence, including cDNA, a gDNA, or a plasmid nucleotide sequence. An amplicon is a short segment of DNA.

Gene quantitation assays using TaqMan® Gene Expression Master Mix and TaqMan® Gene Expression Assays assays are performed in a two-step RT–PCR.

1. In the reverse transcription (RT) step, cDNA is reverse transcribed from RNA.
2. In the PCR step, PCR products are quantitatively synthesized from cDNA samples using the TaqMan® Gene Expression Master Mix.

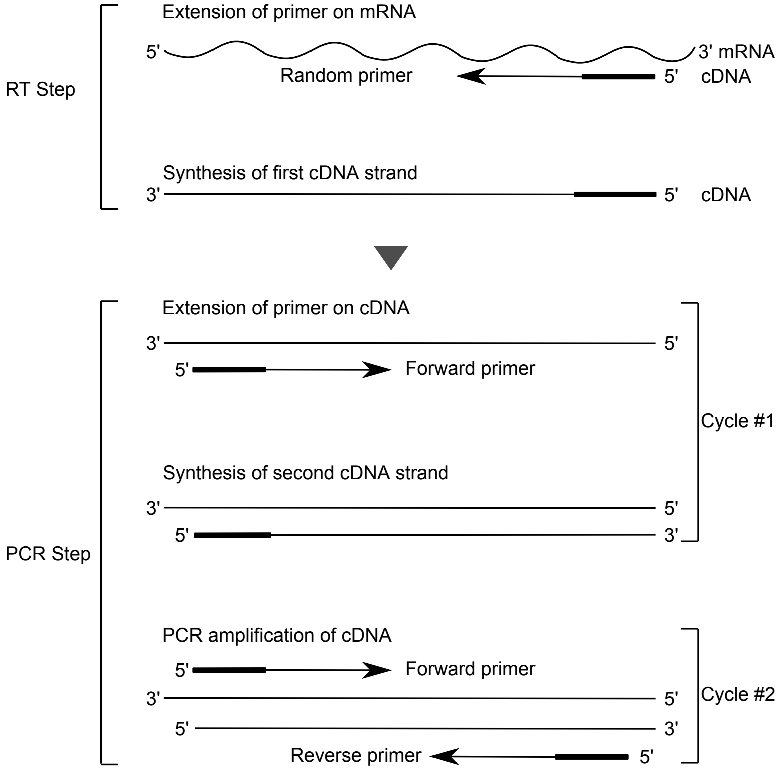

Figure 1 Two–step RT–PCR

This illustration does not show hybridization of the TaqMan® MGB probe. See "TaqMan® MGB probes" on page 30 for details on how the TaqMan® MGB probe is used in the PCR step.

Overview of TaqMan® Gene Expression Assays

TaqMan® MGB probes

TaqMan® MGB probes contain:

- A reporter dye (for example, FAM™) at the 5′ end of the probe.
- A non-fluorescent quencher (NFQ) dye at the 3′ end of the probe.
  The NFQ dye does not fluoresce, which allows the real-time PCR system to measure the reporter dye contributions more accurately.
- A minor groove binder (MGB) at the 3′ end of the probe that:
  - Increases the melting temperature (T_m) without increasing the probe length.
  - Allows for the design of shorter probes.

About the 5' nuclease assay

The 5' nuclease assay process takes place during PCR amplification. It occurs in every cycle and does not interfere with the exponential accumulation of product.

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

When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Förster–type energy transfer.

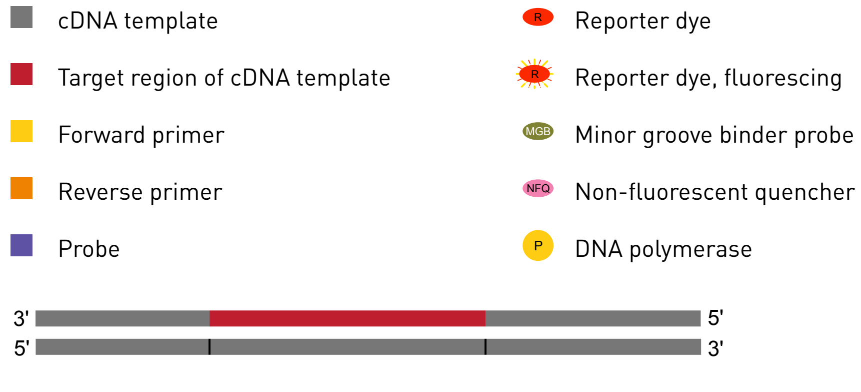

Figure 2 cDNA synthesis product

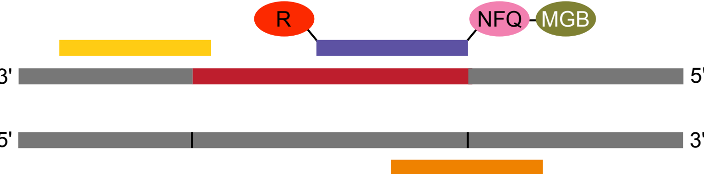

Figure 3 Denature and anneal

The DNA polymerase cleaves only probes that hybridize to the target. Cleavage separates the reporter dye from the quencher dye. This results in increased fluorescence by the reporter. The increase in fluorescence occurs only if the target

sequence is complementary to the probe and amplified during PCR. Because of these requirements, nonspecific amplification is not detected.

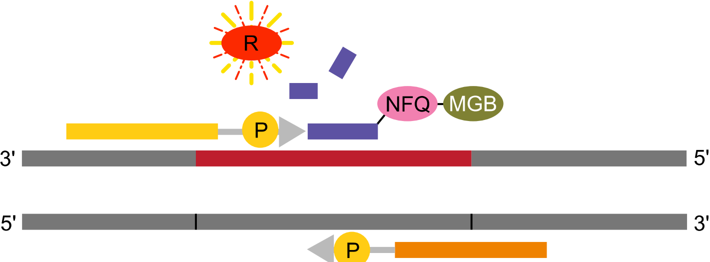

Figure 4 Cleavage

Polymerization of the strand continues. However, no extension of the probe occurs during PCR because the 3' end of the probe is blocked.

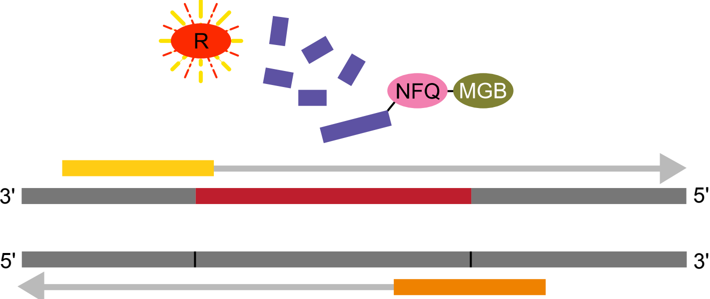

Figure 5 Completion of polymerization

Dyes for multicomponent analysis

Multicomponent analysis is the term that is used to distinguish the contribution that each dye makes to the fluorescent spectra. The combination of spectra from the pure dye components generates the composite spectrum. This spectrum represents one fluorescent reading from one well. The following table lists the dyes available for multicomponent analysis.

Type of dye	Name of dye
Reporter	6-FAM™ dye, Cy3™ dye, Cy5™ dye, JOE™ dye, NED™ dye, TET™ dye, VIC™ dye
Quencher	TAMRA™ dye, QSY™ dye, MGB/NFQ
Passive reference	ROX™ dye, MUSTANG PURPLE™ dye

How to calculate R_n and ΔR_nValues

The R_n is calculated by dividing the emission intensity of the reporter dye by the emission intensity of the ROX™ Passive Reference for a given reaction tube.

R_n^+ is the R_n value of a reaction containing all components including the template.

R_n^- is the R_n value of an unreacted sample. This value can be obtained from the early cycles of a real-time run, or from a reaction not containing template.

ΔR_n is the difference between the R_n^+ value and the R_n^- value. It indicates the magnitude of the signal generated.

The following equation expresses the relationship of these terms:

$\Delta R_n = (R_n^+)-(R_n^-)$

where:

$$R_n^+ = \frac{\text{Emission Intensity of Reporter}}{\text{Emission Intensity of Passive Reference}}$$

PCR with template

$$R_n^- = \frac{\text{Emission Intensity of Reporter}}{\text{Emission Intensity of Passive Reference}}$$

PCR without template or early cycles of a real-time reaction

Guidelines for duplex reactions using TaqMan® Gene Expression Assays

Duplex real-time PCR is the simultaneous amplification and measurement of two target sequences in one reaction. TaqMan® Gene Expression Assays can be used in duplex real-time PCR when using a FAM™ dye-labeled assay in combination with a primer-limited, VIC™ dye-labeled assay. Use the following guidlines for duplex reactions:

- Verify that your duplex assay combinations provide similar results to your singleplex reactions.
- Consider the relative expression levels of each target.
- Perform serial dilutions of your sample in both singleplex and duplex reactions, and compare the results for relative expression.
- Select the higher-expressing target as the primer-limited, VIC™ dye-labeled assay.

For more details on how to validate your duplex assay reactions and interpret the results, see *TaqMan® Assay Multiplex PCR Optimization User Guide* (Pub. No. MAN0010189) or go to **thermofisher.com/multiplexqpcr**.

Best practices for PCR and RT-PCR experiments

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Use UNG to prevent false-positive amplification

Carryover amplicons can result in false-positive amplification during PCR. Use a Master Mix that contains uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)) to degrade many contaminating carryover amplicons.

UNG enzymatic activity occurs during an initial incubation at 50°C. UNG is partially inactivated during the 95°C incubation step for template denaturation and polymerase activation. Because UNG is not completely deactivated during the 95°C incubation, it is important to keep the annealing temperatures greater than 55°C and to refrigerate PCR products at 2°C to 8°C in order to prevent amplicon degradation.

To ensure the desired UNG activity:

- Use PCR components and thermal cycling conditions as specified.

  UNG-containing Master Mixes incorporate the optimal concentration of UNG to prevent cross-contamination while not affecting real-time PCR performance.
- Do not attempt to use UNG-containing Master Mixes in subsequent amplification of dU-containing PCR products, such as in nested-PCR protocols. The UNG will degrade the dU-containing PCR products, preventing further amplification.

Although treatment with UNG can degrade or eliminate large numbers of carryover PCR products, use good laboratory practices to minimize cross-contamination from non-dU-containing PCR products or other samples.

Detect fluorescent contaminants

Fluorescent contaminants can generate false positive results. To help detect these contaminants, we recommend including a no-amplification control reaction that contains sample, but no Master Mix.

After PCR, if the absolute fluorescence of the no-amplification control is greater than the fluorescence of the no template control (NTC), fluorescent contaminants may be present in the sample or in the heat block of the real-time PCR instrument.

Safety

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

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

Chemical safety

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

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
TaqMan® Gene Expression Master Mix Quick Reference Card	4371134
TaqMan® Gene Expression Assays User Guide—single-tube assays	4333458
TaqMan® Gene Expression Assays User Guide—TaqMan® Array Plates	4391016
TaqMan® Gene Expression Assays User Guide—TaqMan® Array Plates	4391016
TaqMan® Gene Expression Assays Quick Reference—96-well Standard (0.2-mL) Plates	4391139
TaqMan® Gene Expression Assays Quick Reference—96-well Fast (0.1-mL) Plates	4427562
TaqMan® Gene Expression Assays User Guide—TaqMan® Array Cards	4400263
TaqMan® Gene Expression Assays Quick Reference—TaqMan® Array Cards	4371129
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Design and Analysis Desktop Software User Guide	MAN0010408
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Getting Started Guide	4489822
QuantStudio™ 12K Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050
StepOne™ Real-Time PCR System	
StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide	4376782

Document	Pub. No.
7500/7500 Fast Real-Time PCR System	
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
7900HT Real-Time PCR Instrument	
Applied Biosystems™ 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide	4351684
Data Analysis	
Real-Time PCR Systems Chemistry Guide: Applied Biosystems™ 7900HT Fast Real-Time PCR System and 7300/7500 Real-Time PCR Systems	4348358
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 Absolute Quantitation Using Standard Curve Getting Started Guide	4364014
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve	4347825
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Quantitation using Comparative C_t	4347824
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785
Applied Biosystems™ Relative Quantitation Analysis Module User Guide	MAN0014820
Applied Biosystems™ Standard Curve Analysis Module User Guide	MAN0014819

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.