

# TaqMan™ Fast Advanced Master Mix

## USER GUIDE

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

**Catalog Numbers** 4444556, 4444557, 4444558, 4444963, 4444964, 4444965

**Publication Number** MAN0025706

**Revision** A.0



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. MAN0025706

Revision	Date	Description
A.0	9 November 2021	<p>Pub. No. 4444605 Rev. D was converted to Pub. No. MAN0025706 Rev. A.0, with the following changes:</p> <ul style="list-style-type: none"><li>• Updated storage temperatures.</li><li>• Added QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems.</li><li>• Added information about recommended algorithms for data analysis.</li><li>• Added instructions to dilute 60X assays, where applicable.</li><li>• Added instructions to divide 20X assays before freezing to avoid multiple freeze-thaw cycles.</li><li>• Added algorithm recommendations for data analysis.</li><li>• Updated polymerase activation time for TaqMan™ Gene Expression Assays (single-tube and TaqMan™ Array Plates).</li><li>• Updated thermal cycling conditions for TaqMan™ Gene Expression Assays and Custom TaqMan™ Gene Expression Assays—single-tube assays.</li><li>• Updated PCR guidelines for TaqMan™ Gene Expression Assays—TaqMan™ Array Cards.</li><li>• Updated anneal/extend temperature for TaqMan™ Gene Expression Assays and TaqMan™ Array Cards on the 7900HT Fast Real-Time PCR Instrument.</li><li>• Removed ramp rates from thermal cycling conditions for TaqMan™ Gene Expression Assays—TaqMan™ Array Cards.</li><li>• Removed use of template files to set up PCR reactions for TaqMan™ Gene Expression Assays—TaqMan™ Array Cards. Added import of setup files and thermal cycling conditions for this format.</li></ul>

**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 trademark of Roche Molecular Systems, Inc., used under permission and license. Microsoft and Excel are trademarks of Microsoft Corporation. AmpliTaq is a registered trademark of Roche Molecular Systems, Inc.

©2021 Thermo Fisher Scientific Inc. All rights reserved.

# Contents

■	<b>CHAPTER 1</b>	<b>Product information</b>	6
		Product description	6
		Purpose of this guide	6
		Contents and storage	7
		Required materials not supplied	7
		Workflow	11
■	<b>CHAPTER 2</b>	<b>RT-PCR for TaqMan™ and Custom TaqMan™ Gene Expression Assays—single-tube assays</b>	12
		Perform reverse transcription	12
		Perform real-time PCR	12
		Guidelines	12
		Before you begin	12
		Prepare the PCR Reaction Mix	13
		Prepare the PCR reaction plate	13
		Set up a plate document or plate file	14
		Run the PCR reaction plate	15
		Analyze data	15
		Algorithms for data analysis	16
■	<b>CHAPTER 3</b>	<b>RT-PCR for TaqMan™ Gene Expression Assays—TaqMan™ Array Plates</b>	17
		Perform reverse transcription	17
		Perform real-time PCR	17
		Guidelines	17
		Before you begin	17
		Prepare the PCR Reaction Mix	18
		Prepare the PCR reaction plate	18
		Set up a plate document or plate file	19
		Run the PCR reaction plate	20
		Analyze data	20
		Algorithms for data analysis	21

- **CHAPTER 4 RT-PCR for TaqMan™ Gene Expression Assays—  
TaqMan™ Array Cards** ..... 22
  - Perform reverse transcription ..... 22
  - Perform real-time PCR ..... 22
    - Guidelines ..... 22
    - Recommended amount of cDNA ..... 23
    - Before you begin ..... 23
    - Prepare the sample-specific PCR Reaction Mix ..... 23
    - Prepare the TaqMan™ Array Card ..... 23
    - Set up a card document or card file ..... 24
    - Run the TaqMan™ Array Card ..... 24
  - Analyze data ..... 25
    - Algorithms for data analysis ..... 25
  
- **CHAPTER 5 RT-PCR for TaqMan™ MicroRNA Assays—single-  
tube assays** ..... 26
  - Perform reverse transcription ..... 26
  - Perform real-time PCR ..... 26
    - Guidelines ..... 26
    - Before you begin ..... 26
    - Prepare the PCR Reaction Mix ..... 27
    - Prepare the PCR reaction plate ..... 27
    - Set up a plate document or experiment file ..... 28
    - Run the PCR reaction plate ..... 29
  - Analyze data ..... 29
    - Algorithms for data analysis ..... 30
  
- **CHAPTER 6 RT-PCR for TaqMan™ Advanced miRNA Assays—single-  
tube assays** ..... 31
  - Perform reverse transcription ..... 31
  - Perform real-time PCR ..... 31
    - Guidelines ..... 31
    - Before you begin ..... 32
    - Prepare PCR Reaction Mix ..... 32
    - Prepare the PCR reaction plate ..... 32
    - Set up a plate document or experiment file ..... 33
    - Run the PCR reaction plate ..... 34
  - Analyze data ..... 34
    - Algorithms for data analysis ..... 35

■	<b>APPENDIX A</b>	<b>Supplemental information</b>	<b>36</b>
		Components of the TaqMan™ Fast Advanced Master Mix	36
		AmpliTaq™ Fast DNA Polymerase	36
		Uracil-N glycosylase	36
		dUTP	36
		ROX™ Passive Reference dye	36
		Two-step real-time RT-PCR	37
		About the 5' nuclease assay	37
		TaqMan™ MGB probes	38
		Enzyme activation time	39
■	<b>APPENDIX B</b>	<b>Best practices for PCR and RT-PCR experiments</b>	<b>40</b>
		Good laboratory practices for PCR and RT-PCR	40
		Use UNG to prevent false-positive amplification	40
		Detect fluorescent contaminants	41
■	<b>APPENDIX C</b>	<b>Safety</b>	<b>42</b>
		Chemical safety	43
		Biological hazard safety	44
■	<b>APPENDIX D</b>	<b>Documentation and support</b>	<b>45</b>
		Customer and technical support	45
		Limited product warranty	45



# Product information

## Product description

The Applied Biosystems™ TaqMan™ Fast Advanced Master Mix enables real-time PCR in any gene expression experiment or quantitative analysis, including the following applications:

- Pathogen detection
- Differential gene expression analysis
- Viral load quantitation
- MicroRNA quantitation
- Microarray verification

The Master Mix can be used with any DNA target, including complementary DNA (cDNA) or genomic DNA (gDNA). It can be used in the second step of a two-step real-time RT-PCR protocol for RNA quantitation experiments. A cDNA template can be generated from RNA using one of our reverse transcription kits prior to real-time PCR with the Master Mix. See details in “Required materials not supplied” on page 7.

The Master Mix is supplied at a 2X concentration and contains the following components:

- AmpliTaq™ Fast DNA Polymerase
- Uracil-N glycosylase (UNG)
- dNTPs with dUTP
- ROX™ Reference Dye (passive reference)
- Optimized buffer components

For more information about each component, see “Components of the TaqMan™ Fast Advanced Master Mix” on page 36.

The Master Mix is optimized for use with primers and TaqMan™ probes designed according to our guidelines.

## Purpose of this guide

This document describes how to perform two-step real-time RT-PCR using TaqMan™ Fast Advanced Master Mix with the following assays or components:

- TaqMan™ Gene Expression Assays (single-tube assays, array plates, and array cards)
  - Single-tube assays
  - TaqMan™ Array Plates
  - TaqMan™ Array Cards
- Custom TaqMan™ Gene Expression Assays

- TaqMan™ MicroRNA Assays
- TaqMan™ Advanced miRNA Assays

This guide provides general guidelines for analyzing data. Analysis can vary between applications. For more information about procedures and data analysis, see the documentation for your instrument.

## Contents and storage

**Table 1** TaqMan™ Fast Advanced Master Mix

Cat. No.	Number of 20- $\mu$ L reactions	Amount	Storage <sup>[1]</sup>
4444556	100	1 $\times$ 1 mL	2–8°C
4444557	500	1 $\times$ 5 mL	
4444963 (2 $\times$ 4444557)	1,000	2 $\times$ 5 mL	
4444964 (5 $\times$ 4444557)	2,500	5 $\times$ 5 mL	
4444965 (10 $\times$ 4444557)	5,000	10 $\times$ 5 mL	
4444558	5,000	1 $\times$ 50 mL	

<sup>[1]</sup> See label for expiration date.

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

**Table 2** Instrument, software, equipment, plates and accessories, and consumables

Item	Source
<b>Instrument, one of the following:</b>	
QuantStudio™ 6 Pro Real-Time PCR System <sup>[1]</sup>	Contact your local sales office.
QuantStudio™ 7 Pro Real-Time PCR System	
QuantStudio™ 3 and 5 Real-Time PCR Instruments <sup>[1]</sup>	
QuantStudio™ 6 Flex Real-Time PCR System <sup>[1]</sup>	
QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
StepOne™ Real-Time PCR System <sup>[2]</sup>	
StepOnePlus™ Real-Time PCR System <sup>[1]</sup>	
7500 Real-Time PCR System <sup>[1]</sup>	

Table 2 Instrument, software, equipment, plates and accessories, and consumables (continued)

Item	Source
7500 Fast Real-Time PCR System <sup>[1]</sup>	Contact your local sales office.
ViiA™ 7 Real-Time PCR System	
7900HT Real-Time PCR Instrument <sup>[1]</sup>	
7900HT Fast Real-Time PCR Instrument	
Compatible real-time PCR instruments from other suppliers may be acceptable. Verify thermal-cycling conditions on other real-time PCR instruments.	
<b>Software</b>	
Microsoft™ Excel™ (Optional, to create plate layout files for import)	<a href="http://microsoft.com">microsoft.com</a>
<b>Equipment</b>	
Centrifuge with plate adapter	MLS
Microcentrifuge	MLS
Thermal cycler, or heat block or water bath set to 95°C	MLS
Adjustable pipettors	MLS
Laboratory mixer (vortex or equivalent)	MLS
<b>Tubes, plates, and other consumables</b>	
Plastics consumables	<a href="http://thermofisher.com/plastics">thermofisher.com/plastics</a>
Pipette tips	<a href="http://thermofisher.com/pipettetips">thermofisher.com/pipettetips</a>
Disposable gloves	MLS

<sup>[1]</sup> Not compatible with TaqMan™ Array Cards.

<sup>[2]</sup> Not compatible with TaqMan™ Array Plates or TaqMan™ Array Cards.

Table 3 Reagents for reverse transcription

Item	Source
<b>Reagents for reverse transcription (all assays)</b>	
TE, pH 8.0, RNase-free	<a href="#">AM9849</a>
(Optional) RNase inhibitor	N8080119 AM2684 (Cloned; 40 U/μL)
Nuclease-Free Water (not DEPC-Treated)	<a href="#">AM9930</a>



Table 3 Reagents for reverse transcription (continued)

Item	Source
<b>Reagents for reverse transcription (TaqMan™ Gene Expression Assays)</b>	
High-Capacity cDNA Reverse Transcription Kit	4368814 4374966 (with RNase inhibitor)
High-Capacity RNA-to-cDNA™ Kit	4387406
SuperScript™ VILO™ cDNA Synthesis Kit	<a href="#">11754050</a>
SuperScript™ IV VILO™ Master Mix	<a href="#">11756050</a>
<b>Reagents for reverse transcription (TaqMan™ MicroRNA Assays)</b>	
TaqMan™ MicroRNA Reverse Transcription Kit <sup>[1]</sup>	4366596
<b>TaqMan™ Advanced miRNA Assays</b>	
TaqMan™ Advanced miRNA cDNA Synthesis Kit <sup>[2]</sup>	<a href="#">A28007</a>

<sup>[1]</sup> TaqMan™ MicroRNA Assays are optimized for use with the TaqMan™ MicroRNA Reverse Transcription Kit. Assay performance cannot be guaranteed with other reverse transcription kits.

<sup>[2]</sup> TaqMan™ Advanced miRNA Assays are optimized for use with the TaqMan™ Advanced miRNA cDNA Synthesis Kit. Assay performance cannot be guaranteed with other reverse transcription kits.

Table 4 Assays

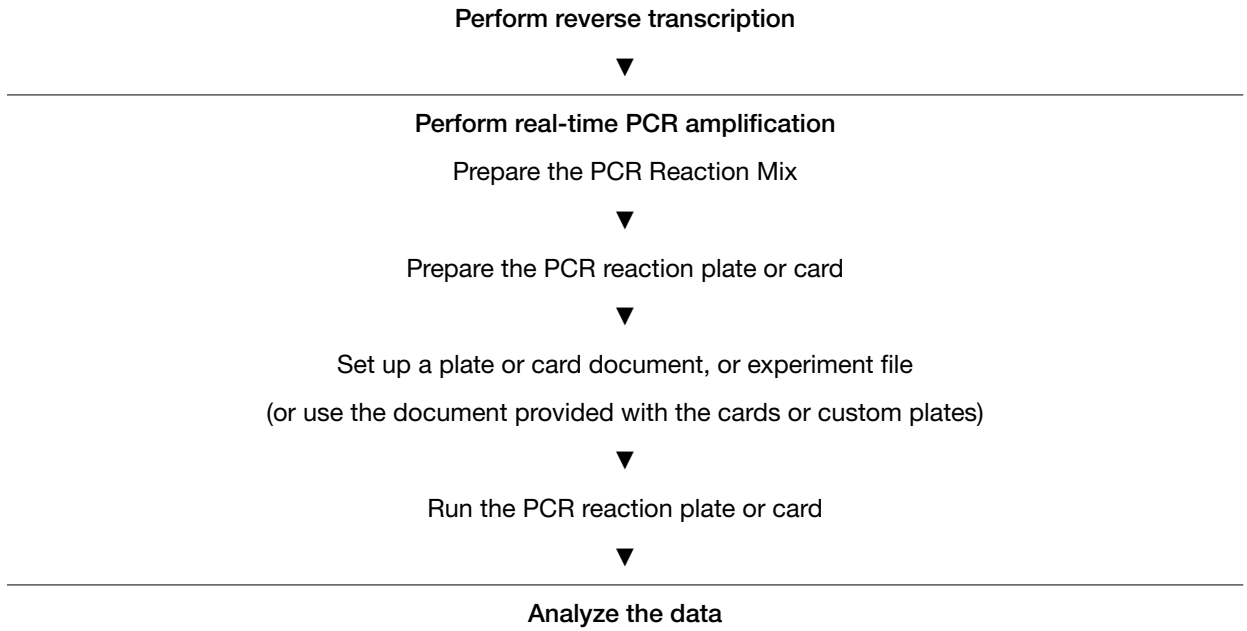
Item	Source
<b>TaqMan™ Assays</b>	
TaqMan™ Gene Expression Assays	<a href="http://thermofisher.com/taqmangeneexpression">thermofisher.com/taqmangeneexpression</a>
Custom TaqMan™ Gene Expression Assays	<a href="http://thermofisher.com/taqmancustomgeneexpression">thermofisher.com/taqmancustomgeneexpression</a>
Custom TaqMan™ probes and primers <sup>[1]</sup>	<a href="http://thermofisher.com/customprimersprobes">thermofisher.com/customprimersprobes</a>
<b>TaqMan™ Array Plates</b>	
96-well Fast (0.1-mL) TaqMan™ Array Plates and 96-well Standard (0.2-mL) TaqMan™ Array Plates	<a href="http://thermofisher.com/taqmanarrays">thermofisher.com/taqmanarrays</a>
<b>TaqMan™ Array Cards</b>	
TaqMan™ Array Card	<a href="http://thermofisher.com/taqmanarrays">thermofisher.com/taqmanarrays</a>
<b>TaqMan™ MicroRNA Assays</b>	
TaqMan™ MicroRNA Assays	<a href="http://thermofisher.com/taqmanmirna">thermofisher.com/taqmanmirna</a>
Custom TaqMan™ Small RNA Assays	<a href="http://thermofisher.com/taqmancustommirna">thermofisher.com/taqmancustommirna</a>
<b>TaqMan™ Advanced miRNA Assays</b>	
TaqMan™ Advanced miRNA Assays	<a href="http://thermofisher.com/advancedmirna">thermofisher.com/advancedmirna</a>

<sup>[1]</sup> Synthesized to your sequence and choice of quencher and reporter dyes.

**Table 5 Kits and reagents for RNA isolation**

Item	Source
RNA isolation products	<a href="https://www.thermofisher.com/rnaisolation">thermofisher.com/rnaisolation</a>
Supporting reagents	<a href="https://www.thermofisher.com/rnaisolationreagents">thermofisher.com/rnaisolationreagents</a>

## Workflow





# RT-PCR for TaqMan™ and Custom TaqMan™ Gene Expression Assays —single-tube assays

## 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 real-time PCR

### Guidelines

- Store the 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.

### Before you begin

Dilute 60X assays to 20X working stocks with TE, pH 8.0, RNase-free.

- Divide the solutions into smaller aliquots to minimize freeze–thaw cycles.

---

**Note:** The aliquot size depends on how many PCR reactions you will run.

---

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

---

**Note:** We recommend four replicate reactions for each assay.

---

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

## Prepare the 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. The minimum final dilution of RT Reaction Mix in PCR Reaction Mix is 1:15.

Component	Volume per reaction		Final concentration
	384-well, 96-well fast (0.1-mL) plates	96-well standard (0.2-mL), 48-well plates	
TaqMan™ Fast Advanced Master Mix (2X)	5.0 µL	10.0 µL	1X
TaqMan™ Assay (20X)	0.5 µL	1.0 µL	1X
Nuclease-free water <sup>[1]</sup>	3.5 µL	7.0 µL	—
<b>Total volume of PCR Reaction Mix per reaction</b>	<b>9.0 µL</b>	<b>18.0 µL</b>	<b>—</b>

<sup>[1]</sup> Adjust the volume of nuclease-free water for a larger volume of cDNA.

2. Vortex briefly to mix.
3. Centrifuge briefly to bring the PCR 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.
2. Add cDNA template (1 pg to 100 ng in nuclease-free water), or nuclease-free water for NTC, to each well.
  - 384-well plate, 96-well fast (0.1 mL) plate: 1.0 µL
  - 96-well standard (0.2 mL) plate, 48-well plate: 2.0 µL

---

**Note:** Adjust the volume of nuclease-free water in the PCR Reaction Mix for a larger volume of cDNA.

---

3. Seal the reaction plate with optical adhesive film, then centrifuge briefly to bring the PCR Reaction Mix to the bottom of the well.
4. Apply a compression pad to the plate, if required by your real-time PCR system.

## Set up a plate document or 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 a plate document or plate file using the following conditions.

Real-time PCR system	(Optional) UNG incubation	Polymerase activation <sup>[1]</sup>	PCR (40 cycles)	
	Hold 50°C	Hold 95°C	Denature 95°C	Anneal / extend 60°C
<ul style="list-style-type: none"> <li>• QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>• QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>• QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>• QuantStudio™ 12K Flex Real-Time PCR System</li> <li>• StepOne™ Real-Time PCR System</li> <li>• StepOnePlus™ Real-Time PCR System</li> <li>• ViiA™ 7 Real-Time PCR System</li> <li>• 7900HT Real-Time PCR System</li> <li>• 7900HT Fast Real-Time PCR System</li> </ul>	2 minutes	20 seconds <sup>[2]</sup>	1 second	20 seconds
<ul style="list-style-type: none"> <li>• 7500 Fast Real-Time PCR System</li> <li>• 7500 Real-Time PCR System</li> </ul>	2 minutes	20 seconds <sup>[2]</sup>	3 seconds	30 seconds

<sup>[1]</sup> To activate AmpliTaq™ Fast DNA Polymerase.

<sup>[2]</sup> Enzyme activation can continue for up to 2 minutes without affecting the results. See “Enzyme activation time” on page 39.

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 a run mode.

Real-time PCR system	Run mode
<ul style="list-style-type: none"> <li>7500 Real-Time PCR System</li> <li>7900HT Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System (384-well and 96-well standard block modules)</li> </ul>	Standard
<ul style="list-style-type: none"> <li>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>QuantStudio™ 12K Flex Real-Time PCR System</li> <li>StepOne™ Real-Time PCR System</li> <li>StepOnePlus™ Real-Time PCR System</li> <li>ViiA™ 7 Real-Time PCR System</li> <li>7500 Fast Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System (384-well and 96-well standard block modules)</li> </ul>	Fast

6. Enter the sample volume, if this option applies to your instrument.

- 384-well plate, 96-well fast (0.1 mL) plate: **10.0 µL**
- 96-well standard (0.2 mL) plate, 48-well plate: **20.0 µL**

## Run the PCR reaction plate

1. Open the plate document or experiment file that corresponds to the reaction plate in the system software.
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 6 Algorithm recommendations for single-tube assays

Algorithm	Recommendation
Threshold ( $C_t$ )	Recommended.
Relative threshold ( $C_{rt}$ )	<i>(Optional)</i> Use for troubleshooting abnormal or unexpected results.

The relative threshold algorithm is available in the Relative Quantification application on Thermo Fisher™ Connect ([thermofisher.com/connect](https://thermofisher.com/connect)).





# RT-PCR for TaqMan™ Gene Expression Assays — TaqMan™ Array Plates

## 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—TaqMan™ Array Plates* (Pub. No. 4391016).

## Perform real-time PCR

### Guidelines

Store the plates away from light until use. Excessive exposure to light might affect the fluorescent probes.

### Before you begin

- Determine the total number of PCR reactions.  
One reaction corresponds to one well in the plate.
- Thaw the Master Mix on ice, then mix thoroughly but gently.
- Thaw the 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 <sup>[1]</sup>	5 µL	10 µL
TaqMan™ Fast Advanced Master Mix (2X)	5 µL	10 µL
<b>Total volume of PCR Reaction Mix per reaction</b>	<b>10 µL</b>	<b>20 µL</b>

<sup>[1]</sup> 5–50 ng of cDNA diluted in nuclease-free water.

2. Vortex briefly to mix.
3. Centrifuge briefly to bring the PCR 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

---

**Note:** Adjust the volume of nuclease-free water in the PCR Reaction Mix for a larger volume of cDNA.

---

2. Seal the plate with optical adhesive film, then centrifuge briefly to bring the PCR Reaction Mix to the bottom of the wells.
3. Apply a compression pad to the plate, if required by your real-time PCR system.

## Set up a plate document or 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. Import the setup file (SDS in TXT format) into the real-time PCR instrument or software.
2. Set up a plate document or plate file using the following conditions.

Real-time PCR system	(Optional) UNG incubation	Polymerase activation <sup>[1]</sup>	PCR (40 cycles)	
	Hold 50°C	Hold 95°C	Denature 95°C	Anneal / extend 60°C
<ul style="list-style-type: none"> <li>• QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>• QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>• QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>• QuantStudio™ 12K Flex Real-Time PCR System</li> <li>• StepOnePlus™ Real-Time PCR System</li> <li>• ViiA™ 7 Real-Time PCR System</li> <li>• 7900HT Real-Time PCR System</li> <li>• 7900HT Fast Real-Time PCR System</li> </ul>	2 minutes	20 seconds <sup>[2]</sup>	1 second	20 seconds
<ul style="list-style-type: none"> <li>• 7500 Fast Real-Time PCR System</li> <li>• 7500 Real-Time PCR System</li> </ul>	2 minutes	20 seconds <sup>[2]</sup>	3 seconds	30 seconds

<sup>[1]</sup> To activate AmpliTaq™ Fast DNA Polymerase.

<sup>[2]</sup> Enzyme activation can continue for up to 2 minutes without affecting the results. See “Enzyme activation time” on page 39.

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 a run mode.

Real-time PCR system	Run mode
<ul style="list-style-type: none"> <li>7500 Real-Time PCR System</li> <li>7900HT Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System</li> </ul>	Standard
<ul style="list-style-type: none"> <li>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>QuantStudio™ 12K Flex Real-Time PCR System</li> <li>StepOnePlus™ Real-Time PCR System</li> <li>ViiA™ 7 Real-Time PCR System</li> <li>7500 Fast Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System (384-well and 96-well standard block modules)</li> </ul>	Fast

## 7. Enter the sample volume, if this option applies to your instrument.

- 96-well fast (0.1 mL) plate: **10.0 µL**
- 96-well standard (0.2 mL) plate: **20.0 µL**

## Run the PCR reaction plate

- Open the plate document or experiment file that corresponds to the reaction plate in the system software.
- Load the reaction plate.
- Start the run.

## Analyze data

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

- View the amplification plots for the reactions.
- 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.
- Use the relative standard curve method or the comparative  $C_t$  method to analyze data.

## Algorithms for data analysis

Table 7 Algorithm recommendations for TaqMan™ Array Plates

Algorithm	Recommendation
Threshold ( $C_t$ )	<ul style="list-style-type: none"><li>Recommended for data analysis.</li></ul>
Relative threshold ( $C_{rt}$ )	<ul style="list-style-type: none"><li><i>(Optional)</i> Use for data analysis.</li><li>Use to troubleshoot unexpected results.</li><li>Use to correct a variable baseline, which can be due to dried-down assays on the plate being reconstituted at different rates.</li></ul>

The relative threshold algorithm is available in the Relative Quantification application on Thermo Fisher™ Connect ([thermofisher.com/connect](https://thermofisher.com/connect)).



# RT-PCR for TaqMan™ Gene Expression Assays – TaqMan™ Array Cards

## Perform reverse transcription

Perform reverse transcription to obtain cDNA from RNA samples.

For reverse transcription kits see Table 3 on page 8. For detailed guidelines and instructions see the protocol for your kit and the *TaqMan™ Gene Expression Assays User Guide – TaqMan™ Array Cards* (Pub. No. 4400263).

## Perform real-time PCR

### Guidelines

- Store the card in its packaging until the packaging has reached room temperature and you are ready to fill it with sample-specific PCR Reaction 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 Reaction Mix made from a single cDNA sample.
- Use 100 µL of sample-specific PCR Reaction 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 Reaction 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 card that will be used for each cDNA sample.
- Thaw the samples on ice, then vortex and briefly centrifuge to resuspend.
- Thaw the Master Mix on ice, then mix thoroughly but gently.

## Prepare the sample-specific 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 <sup>[1]</sup>	50 μL
TaqMan™ Fast Advanced Master Mix (2X)	50 μL
<b>Total volume</b>	<b>100 μL</b>

<sup>[1]</sup> See “Recommended amount of cDNA” on page 23.

2. Vortex briefly to mix.
3. Centrifuge briefly to bring the PCR Reaction Mix to the bottom of the tube.

## Prepare the TaqMan™ Array Card

Fill the 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 document or card 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. Import the setup file (TXT format) into the real-time PCR instrument or software.
2. Set up a card document or card file.

---

**Note:** Thermal cycling conditions depend on the instrument.

---

**Table 8** ViiA™ 7 Real-Time PCR System and compatible QuantStudio™ systems with fast cycling mode

(Optional) UNG incubation	Polymerase activation <sup>[1]</sup>	PCR (40 cycles)	
Hold 50°C	Hold 92°C	Denature 95°C	Anneal / extend 60°C
2 minutes	10 minutes <sup>[2]</sup>	1 second	20 seconds

<sup>[1]</sup> To activate AmpliTaq™ Fast DNA Polymerase.

<sup>[2]</sup> To completely dissolve the primers and probes on the card.

---

**IMPORTANT!** Do not use the default Fast settings.

---

**Table 9** 7900HT Fast Real-Time PCR Instrument with standard cycling mode

(Optional) UNG incubation	Polymerase activation <sup>[1]</sup>	PCR (40 cycles)	
Hold 50°C	Hold 92°C	Denature 97°C	Anneal / extend 60°C
2 minutes	10 minutes <sup>[2]</sup>	1 second	20 seconds

<sup>[1]</sup> To activate AmpliTaq™ Fast DNA Polymerase.

<sup>[2]</sup> To completely dissolve the primers and probes on the card.

## Run the TaqMan™ Array Card

1. Open the card document or experiment file that corresponds to the card in the system software.
2. Load the card.
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 10 Algorithm recommendations for TaqMan™ Array Cards

Algorithm	Recommendation
Relative threshold ( $C_{rt}$ )	Recommended for the following instruments: <ul style="list-style-type: none"><li>• QuantStudio™ Real-Time PCR Instruments</li><li>• ViiA™ 7 instrument</li></ul> 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 Thermo Fisher™ Connect ([thermofisher.com/connect](https://www.thermofisher.com/connect)).



# RT-PCR for TaqMan™ MicroRNA Assays – single-tube assays

This chapter covers use of TaqMan™ MicroRNA Assays. TaqMan™ MicroRNA Assays are also available in the following formats:

- TaqMan™ Array Cards
- TaqMan™ OpenArray™ Plates

For use of TaqMan™ Advanced miRNA Assays see Chapter 6, “RT-PCR for TaqMan™ Advanced miRNA Assays – single-tube assays”.

See [thermofisher.com/taqmanmirna](http://thermofisher.com/taqmanmirna) for more information.

## Perform reverse transcription

Perform reverse transcription to obtain cDNA from RNA samples.

Use the TaqMan™ MicroRNA Reverse Transcription Kit. For detailed guidelines and instructions see *TaqMan™ Small RNA Assay User Guide* (Pub. No. 4364031).

## Perform real-time PCR

### Guidelines

- Store the assays at –20°C.
- Protect the assays from light until use. Excessive exposure to light might affect the fluorescent probes.
- Prepare the PCR Reaction Mix before transferring it to the reaction plate for thermal cycling.

### Before you begin

- Divide 20X assays into smaller aliquots to minimize freeze–thaw cycles. The size of the aliquots depends upon the number of PCR reactions you typically run.
- Determine the total number of PCR reactions required, including a microRNA assay for each cDNA sample, endogenous control assays, and a no-template control (NTC) for each assay.

---

**Note:** We recommend four replicate reactions for each assay.

---

- Thaw the Master Mix on ice, then mix thoroughly but gently.

## Prepare the PCR Reaction Mix

- Combine the following components for the number of reactions required.

Add 10% overage for pipetting loss.

Component	Volume per reaction	
	384-well, 96-well fast (0.1-mL) plates	96-well standard (0.2-mL), 48-well plates
TaqMan™ Fast Advanced Master Mix (2X)	5.00 µL	10.00 µL
Nuclease-free water <sup>[1]</sup>	3.83 µL	7.67 µL
TaqMan™ MicroRNA Assay (20X)	0.50 µL	1.00 µL
cDNA template <sup>[2]</sup>	0.67 µL	1.33 µL
<b>Total volume of PCR Reaction Mix per reaction</b>	<b>10.00 µL</b>	<b>20.00 µL</b>

<sup>[1]</sup> Adjust the volume of nuclease-free water for a larger volume of cDNA.

<sup>[2]</sup> The minimum final dilution of RT reaction in PCR reaction is 1:15.

- Mix gently, then centrifuge to bring the PCR Reaction Mix to the bottom of the tube.

## Prepare the PCR reaction plate

- Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.
  - 384-well plate, 96-well fast (0.1 mL) plate: 10 µL
  - 48-well plate, 96-well standard (0.2 mL) plate: 20 µL

---

**Note:** Adjust the volume of nuclease-free water in the PCR Reaction Mix for a larger volume of cDNA.

---

- Seal the plate with optical adhesive film, then centrifuge briefly to bring the PCR Reaction Mix to the bottom of the wells.
- 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.

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

1. Set up a plate document or plate file using the following conditions.

Real-time PCR system	Polymerase activation <sup>[1]</sup>	PCR (40 cycles)	
	Hold 95°C	Denature 95°C	Anneal / extend 60°C
<ul style="list-style-type: none"> <li>• QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>• QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>• QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>• QuantStudio™ 12K Flex Real-Time PCR System</li> <li>• StepOne™ Real-Time PCR System</li> <li>• StepOnePlus™ Real-Time PCR System</li> <li>• ViiA™ 7 Real-Time PCR System</li> <li>• 7900HT Real-Time PCR System</li> <li>• 7900HT Fast Real-Time PCR System</li> </ul>	20 seconds	1 second	20 seconds
<ul style="list-style-type: none"> <li>• 7500 Real-Time PCR System</li> <li>• 7500 Fast Real-Time PCR System</li> </ul>	20 seconds	3 seconds	30 seconds

<sup>[1]</sup> To activate AmpliTaq™ Fast DNA Polymerase.

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 a run mode.

Real-time PCR system	Run mode
<ul style="list-style-type: none"> <li>7500 Real-Time PCR System</li> <li>7900HT Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System (384-well and 96-well standard block modules)</li> </ul>	Standard
<ul style="list-style-type: none"> <li>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>QuantStudio™ 12K Flex Real-Time PCR System</li> <li>StepOne™ Real-Time PCR System</li> <li>StepOnePlus™ Real-Time PCR System</li> <li>ViiA™ 7 Real-Time PCR System</li> <li>7500 Fast Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System (384-well and 96-well standard block modules)</li> </ul>	Fast

6. Enter the sample volume, if this option applies to your instrument.

- 384-well plate, 96-well fast (0.1 mL) plate: **10.0 µL**
- 96-well standard (0.2 mL) plate, 48-well plate: **20.0 µL**

## Run the PCR reaction plate

1. Open the plate document or experiment file that corresponds to the reaction plate in the system software.
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 11 Algorithm recommendations for single-tube assays

Algorithm	Recommendation
Threshold ( $C_t$ )	Recommended.
Relative threshold ( $C_{rt}$ )	<i>(Optional)</i> Use for troubleshooting abnormal or unexpected results.

The relative threshold algorithm is available in the Relative Quantification application on Thermo Fisher™ Connect ([thermofisher.com/connect](https://thermofisher.com/connect)).



# RT-PCR for TaqMan™ Advanced miRNA Assays—single-tube assays

This chapter covers use of TaqMan™ Advanced miRNA Assays. For use of TaqMan™ MicroRNA Assays see Chapter 5, “RT-PCR for TaqMan™ MicroRNA Assays—single-tube assays”.

TaqMan™ Advanced miRNA Assays are also available in the following formats:

- TaqMan™ Array Plates
- TaqMan™ Array Cards
- TaqMan™ OpenArray™ Plates  
(TaqMan™ OpenArray™ Real-Time PCR Master Mix is recommended for TaqMan™ OpenArray™ Plates).

See [thermofisher.com/advancedmirna](http://thermofisher.com/advancedmirna) for more information.

For details about predefined TaqMan™ Advanced miRNA Assays see *TaqMan™ Advanced miRNA Assays User Guide—TaqMan™ Array Plates* (Pub. No. MAN0016120).

## Perform reverse transcription

Use the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Cat. No. [A28007](#)). For detailed guidelines and instructions see *TaqMan™ Advanced miRNA Assays User Guide—Single-tube Assays* (Pub. No. 100027897).

Prepare cDNA templates with the following reactions:

- Poly(A) tailing reaction
- Adaptor ligation reaction
- Reverse transcription reaction
- miR-Amp reaction

## Perform real-time PCR

### Guidelines

- Store the assays at –20°C.
- Protect the assays from light until use. Excessive exposure to light might affect the fluorescent probes.
- Prepare the PCR Reaction Mix before transferring it to the reaction plate for thermal cycling.

## Before you begin

- Divide 20X assays into smaller aliquots to minimize freeze–thaw cycles. The size of the aliquots depends upon the number of PCR reactions you typically run.
- Determine the total number of PCR reactions required, including a microRNA assay for each cDNA sample, endogenous control assays, and a no-template control (NTC) for each assay.

---

**Note:** We recommend four replicate reactions for each assay.

---

- Thaw the Master Mix on ice, then mix thoroughly but gently.

## Prepare PCR Reaction Mix

1. Prepare 1:10 dilutions of the cDNA template.  
For example, add 5 µL of the miR-Amp reaction product to 45 µL of 0.1X TE buffer.
2. Combine the following components for the number of reactions required.  
Add 10% overage for pipetting loss.

Component	Volume per reaction	
	384-well, 96-well fast (0.1–mL) plates	96-well standard (0.2-mL), 48-well plates
TaqMan™ Fast Advanced Master Mix (2X)	5.00 µL	10.00 µL
Nuclease-free water <sup>[1]</sup>	2.00 µL	4.00 µL
TaqMan™ Advanced miRNA Assay (20X)	0.50 µL	1.00 µL
cDNA template (1:10 dilution)	2.50 µL	5.00 µL
<b>Total volume per reaction</b>	<b>10.00 µL</b>	<b>20.00 µL</b>

<sup>[1]</sup> Adjust the volume of nuclease-free water for a larger volume of cDNA.

3. Mix gently, then centrifuge to bring the PCR 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 an optical reaction plate.
  - 384-well plate, 96-well fast (0.1 mL) plate: 10 µL
  - 48-well plate, 96-well standard (0.2 mL) plate: 20 µL

---

**Note:** Adjust the volume of nuclease-free water in the PCR Reaction Mix for a larger volume of cDNA.

---



2. Seal the plate with optical adhesive film, then centrifuge briefly to bring the PCR Reaction Mix to the bottom of the wells.
3. 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.

---

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

---

1. Set up a plate document or plate file using the following conditions.

Real-time PCR system	Polymerase activation <sup>[1]</sup>	PCR (40 cycles)	
	Hold 95°C	Denature 95°C	Anneal / extend 60°C
<ul style="list-style-type: none"> <li>• QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>• QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>• QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>• QuantStudio™ 12K Flex Real-Time PCR System</li> <li>• StepOne™ Real-Time PCR System</li> <li>• StepOnePlus™ Real-Time PCR System</li> <li>• ViiA™ 7 Real-Time PCR System</li> <li>• 7900HT Real-Time PCR System</li> <li>• 7900HT Fast Real-Time PCR System</li> </ul>	20 seconds	1 second	20 seconds
<ul style="list-style-type: none"> <li>• 7500 Real-Time PCR System</li> <li>• 7500 Fast Real-Time PCR System</li> </ul>	20 seconds	3 seconds	30 seconds

<sup>[1]</sup> To activate AmpliTaq™ Fast DNA Polymerase.

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 a run mode.

Real-time PCR system	Run mode
<ul style="list-style-type: none"> <li>7500 Real-Time PCR System</li> <li>7900HT Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System (384-well and 96-well standard block modules)</li> </ul>	Standard
<ul style="list-style-type: none"> <li>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</li> <li>QuantStudio™ 3/QuantStudio™ 5 Flex Real-Time PCR System</li> <li>QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</li> <li>QuantStudio™ 12K Flex Real-Time PCR System</li> <li>StepOne™ Real-Time PCR System</li> <li>StepOnePlus™ Real-Time PCR System</li> <li>ViiA™ 7 Real-Time PCR System</li> <li>7500 Fast Real-Time PCR System</li> <li>7900HT Fast Real-Time PCR System (384-well and 96-well standard block modules)</li> </ul>	Fast

## 6. Enter the sample volume, if this option applies to your instrument.

- 384-well plate, 96-well fast (0.1 mL) plate: **10.0 µL**
- 96-well standard (0.2 mL) plate, 48-well plate: **20.0 µL**

## Run the PCR reaction plate

1. Open the plate document or experiment file that corresponds to the reaction plate in the system software.
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 setting and threshold setting of 0.1, 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 12 Algorithm recommendations for single-tube assays

Algorithm	Recommendation
Threshold ( $C_t$ )	Recommended.
Relative threshold ( $C_{rt}$ )	<i>(Optional)</i> Use for troubleshooting abnormal or unexpected results.

The relative threshold algorithm is available in the Relative Quantification application on Thermo Fisher™ Connect ([thermofisher.com/connect](https://thermofisher.com/connect)).



# Supplemental information

## Components of the TaqMan™ Fast Advanced Master Mix

### AmpliTaq™ Fast DNA Polymerase

The AmpliTaq™ Fast DNA Polymerase enzyme is purified through a proprietary process to reduce bacterial DNA introduced from the host organism. The purification process ensures that non-specific, false-positive DNA products due to bacterial DNA contamination are minimized during PCR.

When AmpliTaq™ Fast DNA Polymerase is added to the reaction mix at room temperature, the inactive enzyme is not capable of primer extension. Any low-stringency mispriming events that may have occurred 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. For more information about UNG see “Use UNG to prevent false-positive amplification” on page 40.

### dUTP

This Master Mix includes dUTP to enable uracil-N-glycosylase (UNG) activity and maintain optimal PCR results.

### 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 real-time RT-PCR

Visit [thermofisher.com/qpcreducation](http://thermofisher.com/qpcreducation) for more information.

**Note:** TaqMan™ MicroRNA Assays and TaqMan™ Advanced miRNA Assays do not use the same RT chemistry.

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 quantification assays using TaqMan™ Fast Advanced Master Mix and TaqMan™ 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 Master Mix.

For details on how the TaqMan™ MGB probe is used in the PCR step, see “TaqMan™ MGB probes” on page 38.

## 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.

These figures do not represent TaqMan™ Advanced miRNA Assays because the RT chemistry is different. However, the principle of the 5' nuclease assay during PCR amplification remains the same. These assays also use a forward primer, a reverse primer, and a probe.

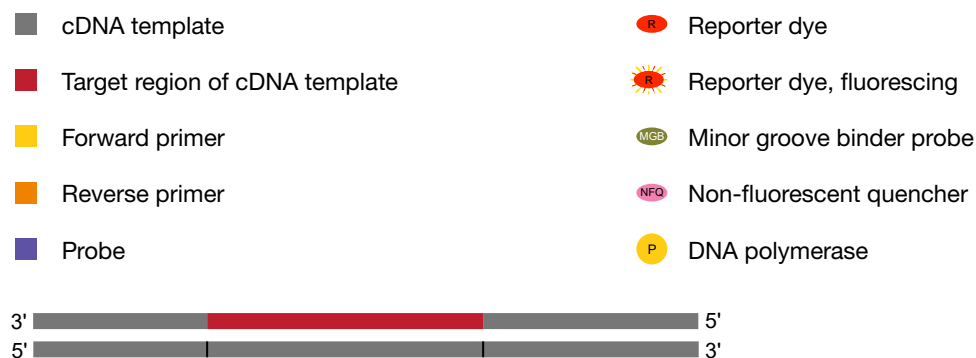
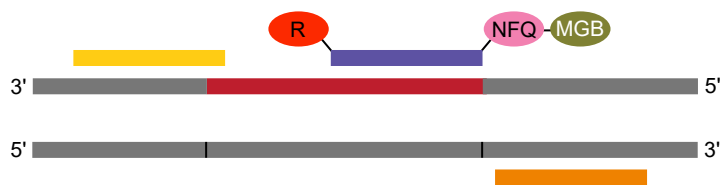
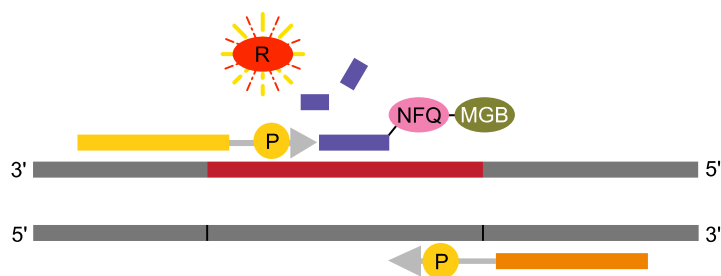


Figure 1 cDNA synthesis product



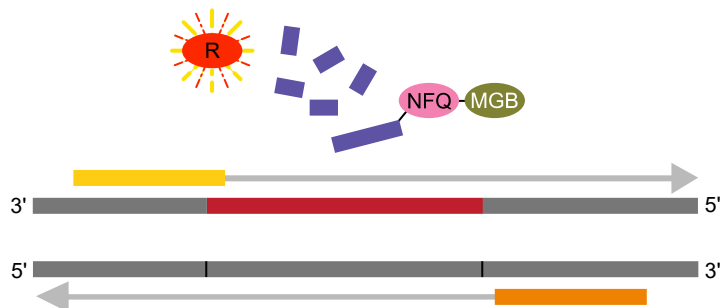
**Figure 2 Denature and anneal**

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 3 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 4 Completion of polymerization**

## TaqMan™ MGB probes

TaqMan™ MGB probes contain:

- A reporter dye (for example, FAM™ dye) 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.

## Enzyme activation time

Using TaqMan™ Fast Advanced Master Mix, the enzyme activation step can range from 20 seconds to 2 minutes. A 20-second enzyme activation step is sufficient when the template is cDNA. A longer enzyme activation time will not affect the results.

The enzyme activation time for the default fast thermal cycling conditions on the instruments is 20 seconds. If a longer enzyme activation time is required, change the thermal cycling conditions before starting the run. A longer enzyme activation time can help to denature double-stranded genomic DNA when genomic DNA is used.



# 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.



**AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.



- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

## Biological hazard safety



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



**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. 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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:  
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf>
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs); found at:  
[www.who.int/publications/i/item/9789240011311](http://www.who.int/publications/i/item/9789240011311)



# Documentation and support

## Customer and technical support

Visit [thermofisher.com/support](http://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](http://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](http://www.thermofisher.com/support).

