

# TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix

## USER GUIDE

For one-step RT-PCR of viral nucleic acid

Catalog Numbers 4444432, 4444434, 4444436

Publication Number 4453800

Revision D



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](http://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. 4453800

Revision	Date	Description
D	12 October 2018	<ul style="list-style-type: none"><li>• Clarified procedures.</li><li>• Updated reaction volumes.</li><li>• Updated compatible instruments.</li><li>• Updated supplies information.</li><li>• Updated storage conditions.</li><li>• Reorganized.</li></ul>
C	May 2012	Baseline for this revision history.

**Important Licensing Information:** This product may be covered by one or more Limited Use Label Licenses. By use of this product, 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.

©2018 Thermo Fisher Scientific Inc. All rights reserved.

# Contents

■	<b>CHAPTER 1</b>	<b>Product information</b>	5
	Product description		5
	Reverse transcriptase enzyme		5
	Order TaqMan <sup>®</sup> Gene Expression Assays		5
	Contents and storage		6
	Required materials not supplied		6
	Workflow		8
■	<b>CHAPTER 2</b>	<b>Guidelines for preparation of nucleic acid</b>	9
	Starting template		9
	Guidelines for preparation of high-quality nucleic acid samples		9
■	<b>CHAPTER 3</b>	<b>Perform RT-PCR</b>	10
	Procedural guidelines		10
	Before you begin (60X assays)		11
	Prepare the RT-PCR Reaction Mix		11
	Set up and run the real-time PCR instrument		13
	Analyze the results		14
■	<b>APPENDIX A</b>	<b>Troubleshooting</b>	15
■	<b>APPENDIX B</b>	<b>Supplemental information</b>	17
	Experiment types		17
	Determine the number of reactions		18
	Determine optimal primer concentrations		19
	Primer concentrations to test		19
	Prepare and run the RT-PCR reactions		20
	Analyze the results		22
	Determine optimal probe concentration		22
	Probe concentrations to test		22
	Prepare and run the RT-PCR reactions		22
	Analyze the results		24

- **APPENDIX C Best practices for PCR and RT-PCR experiments .... 25**
  - Good laboratory practices for PCR and RT-PCR ..... 25
  - Detect fluorescent contaminants ..... 25
- **APPENDIX D Safety ..... 26**
  - Chemical safety ..... 27
  - Biological hazard safety ..... 28
  - Documentation and support ..... 29**
    - Related documentation ..... 29
    - Customer and technical support ..... 30
    - Limited product warranty ..... 31



# Product information

■ Product description .....	5
■ Required materials not supplied .....	6
■ Workflow .....	8

---

**IMPORTANT!** Before using this product, read and understand the information in the “Safety” appendix in this document.

---

## Product description

The Applied Biosystems™ TaqMan® Fast Virus 1-Step Master Mix can be used with any TaqMan® primer and probe set for RNA or DNA virus research. During thermal cycling, the reverse transcription step does not affect performance with DNA targets.

The Master Mix is supplied at a 4X concentration and contains:

- AmpliTaq™ Fast DNA Polymerase
- Thermostable MMLV enzyme
- dNTPs including dATP, dGTP, dCTP, and dTTP
- RNaseOUT™ Recombinant Ribonuclease Inhibitor
- ROX™ dye (passive reference)
- Buffer components optimized for maximum sensitivity and tolerance to several common RT-PCR inhibitors

The TaqMan® Fast Virus 1-Step Master Mix enables one-step RT-PCR for presence/absence, standard curve, and relative quantification experiments. For details on experiment types, see “Experiment types” on page 17.

### Reverse transcriptase enzyme

The reverse transcriptase enzyme contained in this kit is produced using an *E. coli* expression vector containing a proprietary version of the MMLV *pol* gene (GenBank accession No. J02255) expressed from pET-24(+). It is possible that a minimal amount of the expression vector could be carried over into the final Master Mix formulation. To target MMLV, a related virus, or any of the plasmid sequence, it is recommended to design primer sequences not contained in the expression vector.

### Order TaqMan® Gene Expression Assays

To order predesigned, preformulated primer and probe sets, go to [thermofisher.com/taqmangeneexpression](http://thermofisher.com/taqmangeneexpression), then use the **Assay Search Tool**.

To order Custom TaqMan® Gene Expression Assays, go to our **Assay Design Tool**. Enter and submit sequences for the new assay design.

See the *Custom TaqMan® Assays Design and Ordering Guide* (Pub. No. 4367671).

## User-designed assays

Use Primer Express™ Software v3.0.1 to design custom assays (primer and probe sets) for use with the TaqMan® Fast Virus 1-Step Master Mix.

## Contents and storage

Cat. No.	Contents	Number of 20-µL reactions	Storage <sup>[1]</sup>
4444432	1 x 1 mL	200	-25°C to -15°C
4444434	5 x 1 mL	1000	
4444436	1 x 10 mL	2000	

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

## Required materials not supplied

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

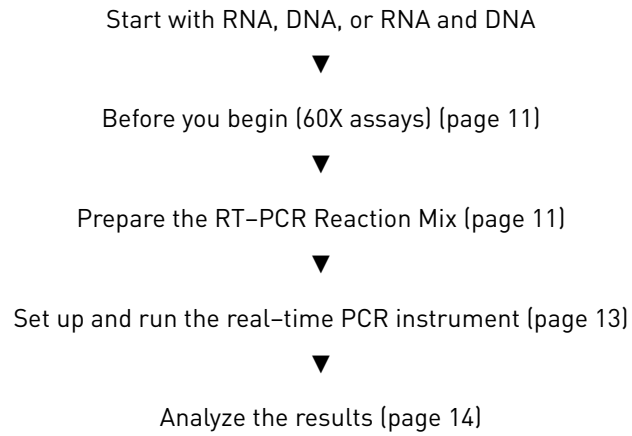
**Table 1** Recommended products for isolation of RNA or DNA

Item	Source
MagMAX™ -96 Viral RNA Isolation Kit	AM1836
MagMAX™ -96 Total RNA Isolation Kit	AM1830
MagMAX™ -96 DNA Multi-Sample Kit	4413021
MagMAX™ Viral RNA Isolation Kit	AM1939
PureLink™ Viral RNA/DNA Mini Kit	12280050
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
RNAqueous™ Total RNA Isolation Kit	AM1912
RNAqueous™ -4PCR Total RNA Isolation Kit	AM1914
TURBO DNA-free™ Kit	AM1907
DNase I, Amplification Grade	18068015
TURBO™ DNase (2 U/µL)	AM2239
RNAlater™ -ICE Frozen Tissue Transition Solution	AM7030
RNAlater™ Stabilization Solution	AM7020
TRI Reagent™ Solution	AM9738

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

Item	Source
<b>Instrument, one of the following:</b>	
QuantStudio™ 3 or 5 Real-Time PCR Instrument	Contact your local sales office.
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
StepOne™ or StepOnePlus™ Real-Time PCR System	
7500/7500 Fast Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
Or use a compatible real-time PCR instrument from another supplier. <b>Note:</b> Verify thermal cycling conditions on other real-time PCR instruments.	
<b>Software</b>	
Primer Express™ Software	4363991
<b>Equipment</b>	
Centrifuge with plate adapter	MLS
Microcentrifuge	MLS
Single- or multi-channel pipettes (electronic or manual)	MLS
Laboratory mixer (vortex or equivalent)	MLS
<b>Tubes, plates, and other consumables</b>	
Tubes, plates and film	<a href="http://thermofisher.com/plastics">thermofisher.com/plastics</a>
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
<b>Reagents</b>	
TE, pH 8.0	AM9849
TaqMan® Gene Expression Assays	<a href="http://thermofisher.com/taqmangeneexpression">thermofisher.com/taqmangeneexpression</a>
Nuclease-free Water (not DEPC-Treated)	AM9930
TaqMan® Exogenous Internal Positive Control Reagents Kit	4308323
RT-PCR Grade Water	AM9935

## Workflow







# Guidelines for preparation of nucleic acid

## Starting template

RT-PCR with TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix can be performed with both DNA and RNA. The reverse transcription step will not affect the DNA targets.

## Guidelines for preparation of high-quality nucleic acid samples

- See “Required materials not supplied” on page 6 for recommended kits to isolate RNA or DNA.
- Store isolated nucleic acid at  $-86^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ .
- The viral RNA isolation kits include carrier RNA to maximize RNA recovery.
- *(Optional)* Use RNAlater<sup>™</sup>-ICE Frozen Tissue Transition Solution when thawing frozen tissue for RNA extraction to preserve the RNA.
- *(Optional)* Use RNAlater<sup>™</sup> Stabilization Solution to stabilize RNA in tissue.



# Perform RT-PCR

- Procedural guidelines ..... 10
- Before you begin (60X assays) ..... 11
- Prepare the RT-PCR Reaction Mix ..... 11
- Set up and run the real-time PCR instrument ..... 13
- Analyze the results ..... 14

## Procedural guidelines

- Protect the assays from light and store as indicated until ready for use. Excessive exposure to light can negatively affect the fluorescent probes of the assays.
- Thaw the assays and Master Mix on ice, then mix thoroughly but gently.  
**Note:** The Master Mix does not freeze at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$ , but gelling can occur. Thawing the Master Mix on ice allows it to return to its liquid state.
- At first use, prepare aliquots of the assays to avoid multiple freeze and thaw cycles.
- Use TE buffer or Nuclease-free Water (not DEPC-Treated) to dilute samples or to prepare the standard dilution series.
- TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix is designed to accommodate multiple assays. For guidelines on designing multiplex reactions, see *TaqMan<sup>®</sup> Multiplex PCR Optimization User Guide* (Pub. No. MAN0010189).
- Verify the assays and optimize the thermal-cycling conditions for assays other than TaqMan<sup>®</sup> Gene Expression Assays or Custom TaqMan<sup>®</sup> Gene Expression Assays, or when using thermal-cycling conditions other than those specified in this protocol.

**Note:** (Optional) TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix and the TaqMan<sup>®</sup> Gene Expression Assay can be combined ahead of time and stored at  $-30^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$  for short periods. The assay can be added directly to the Master Mix tubes. The volumes in shipped tubes are as precise as a pipette. The target fill volume for the 1-mL tubes is 1.05 mL. The target fill volume for the 10-mL tubes is 10.3 mL.

## Before you begin (60X assays)

Dilute 60X assays to 20X working stocks with TE, pH 8.0, then divide the solutions into smaller aliquots to minimize freeze-thaw cycles. The size of the aliquots depends upon the number of PCR reactions you typically run. An example dilution is shown in the following table.

1. Gently vortex the tube of 60X assay, then centrifuge briefly to spin down the contents and eliminate air bubbles.
2. In a 1.5-mL microcentrifuge tube, dilute sufficient amounts of 60X assay for the required number of reactions. For details, see “Determine the number of reactions” on page 18.

Component	Volume
TaqMan® Gene Expression Assays (60X) or Custom TaqMan® Gene Expression Assays (60X)	40 µL
TE, pH 8.0 (1X)	80 µL
<b>Total aliquot volume</b>	<b>120 µL</b>

3. Store aliquots at –20°C until use.

## Prepare the RT-PCR Reaction Mix

Thaw the reagents and nucleic acid samples on ice. Resuspend the nucleic acid samples by inverting the tube, then gently vortexing.

1. Mix the TaqMan® Fast Virus 1-Step Master Mix thoroughly but gently until homogenous.
2. Prepare the RT-PCR Reaction Mix for the number of reactions required as shown in table below, plus 10% overage.

Component	Volume per well	
	384-well Plate or 96-well Fast (0.1-mL) Plate	96-well Standard (0.2-mL) Plate
TaqMan® Fast Virus 1-Step Master Mix	5 µL	12.5 µL
TaqMan® Gene Expression Assay or Custom TaqMan® Gene Expression Assay (20X) <sup>[1]</sup>	1 µL	2.5 µL
RT-PCR Grade Water	Varies <sup>[2]</sup>	Varies <sup>[3]</sup>
<b>Total RT-PCR Reaction Mix volume per reaction</b>	<b>20 µL</b>	<b>50 µL</b>

<sup>[1]</sup> If you are not using preformulated TaqMan® Gene Expression Assays, we recommend primer concentrations of 400–900 nM and a probe concentration of 100–250 nM.

<sup>[2]</sup> Sample volume will vary depending on experiment type. Add sufficient water to bring total to 20 µL *after* accounting for sample volume.

<sup>[3]</sup> Sample volume will vary depending on experiment type. Add sufficient water to bring total to 50 µL *after* accounting for sample volume.

3. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

---

**IMPORTANT!** The Master Mix is viscous because it is at 4X concentration. Ensure that all components are mixed thoroughly.

---

4. Transfer the RT-PCR Reaction Mix to the appropriate wells of a reaction plate.

**Note:** These volumes are recommended when working with viruses, as larger volumes are typically required to detect the low abundant virus. For targets present in high abundance, total volume can be decreased to 10  $\mu$ L for the 384-well Plate or 96-well Fast (0.1-mL) Plate and 20  $\mu$ L for the 96-well Standard (0.2-mL) Plate.

5. Add the following amounts of sample nucleic acid to the reaction plate wells.

- 384-well Plate or 96-well Fast (0.1-mL) Plate: 1 pg to 100 ng
- 96-well Standard (0.2-mL) Plate: 1 pg to 100 ng

**Note:** Do not use more than 1  $\mu$ g of sample.

6. Seal the plate with an optical adhesive cover, then vortex briefly or invert the plate to mix the contents.

**Note:** Invert the plate for more uniform mixing because the Master Mix is viscous.

7. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

## Set up and run the real-time PCR instrument

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

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

1. Select the appropriate cycling mode.  
TaqMan® Fast Virus 1-Step Master Mix is compatible with Fast or Standard cycling mode.
2. Set up the thermal protocol.

**Table 3** Standard cycling mode (reaction volume >30 µL)

Step	Temperature	Time	Cycles
Reverse transcription <sup>[1]</sup>	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	15 seconds	40
Anneal / extend <sup>[2]</sup>	60°C	60 seconds	

<sup>[1]</sup> RT enzyme will function best in the range of 48–55°C.

<sup>[2]</sup> Ensure that the annealing temperature is consistent with the melting temperature ( $T_m$ ) of your primer designs.

**Table 4** Fast cycling mode (reaction volume <30 µL)

Step	Temperature	Time	Cycles
Reverse transcription <sup>[1]</sup>	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / extend <sup>[2]</sup>	60°C	30 seconds	

<sup>[1]</sup> RT enzyme will function best in the range of 48–55°C.

<sup>[2]</sup> Ensure that the annealing temperature is consistent with the melting temperature ( $T_m$ ) of your primer designs.

3. Set the reaction volume appropriate for the reaction plate.
  - 96-well Standard (0.2-mL) Plate: **50 µL**
  - 384-well Plate or 96-well Fast (0.1-mL) Plate: **20 µL**
4. Load the plate into the real-time PCR instrument.
5. Start the run.

## Analyze the results

For more information about data analysis, see the appropriate documentation for your assay and instrument. Use the standard curve method or the relative quantification ( $\Delta\Delta C_t$ ) method to analyze results.

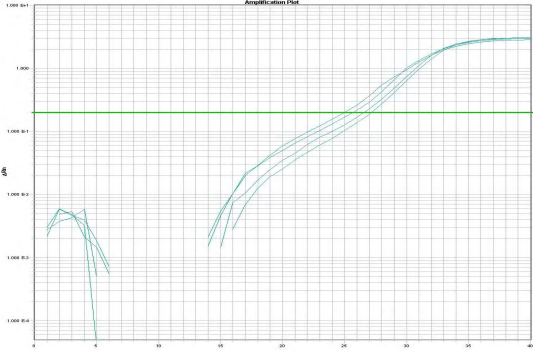
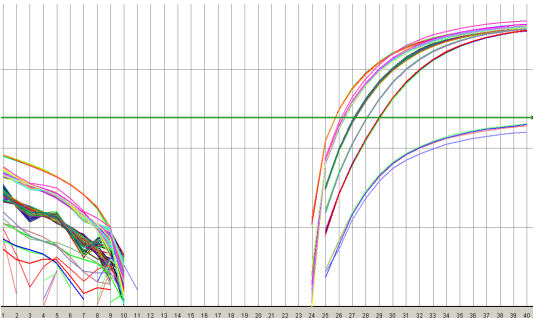
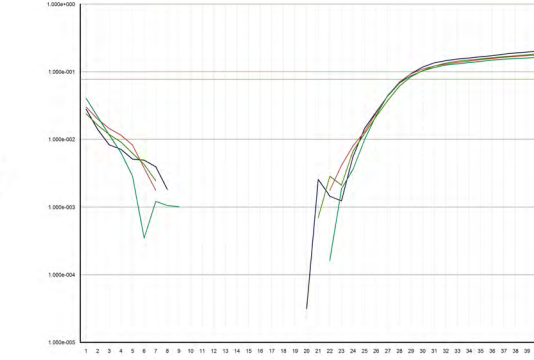
The general guidelines for analysis include:

- View the amplification plot. Then, if needed:
  - Adjust the baseline and threshold values.
  - Review replicates and outliers.
- In the well table or results table, view the  $C_t$  values for each well and for each replicate group.

For more information about real-time PCR, see *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239) or go to [www.thermofisher.com/qpcducation](http://www.thermofisher.com/qpcducation).



# Troubleshooting

Observation	Possible cause	Recommended action
<p>The amplification plot displays S-shaped curves</p> 	<p>The RT-PCR Reaction Mix was not thoroughly combined.</p>	<p>Repeat the assay. Follow the instructions for mixing the reagents (see “Prepare the RT-PCR Reaction Mix” on page 11).</p>
<p>The amplification plot is truncated</p> 	<p>The baseline was set too high.</p>	<p>Reset the lower value of the baseline range. Use an automatic baseline.</p>
<p>The <math>C_t</math> is low and merges with the background signal</p> 	<p>When automatic baseline is used, the software raises the threshold bar to avoid the elevated baseline.</p>	<p>Use a manual <math>C_t</math>, then manually adjust the threshold.</p>



Observation	Possible cause	Recommended action
There is unexpected amplification	There was amplification of genomic DNA.	Run a no-RT control reaction to confirm that there was genomic DNA contamination.  <ol style="list-style-type: none"><li>1. Inactivate the RT enzyme by incubating Master Mix at 95°C for 5 minutes.</li><li>2. Allow the Master Mix to cool to room temperature.</li></ol> <p><b>Note:</b> The PCR hot-start mechanism will reactivate.</p>
		<i>{ Custom TaqMan® Gene Expression Assays only }</i> Design an assay that spans an exon-exon boundary. See <i>Custom TaqMan® Assays Design and Ordering Guide</i> (Pub. No. 4367671).
		Treat the RNA sample with DNase I.





# Supplemental information

■ Experiment types .....	17
■ Determine the number of reactions .....	18
■ Determine optimal primer concentrations .....	19
■ Determine optimal probe concentration .....	22

## Experiment types

The Applied Biosystems™ TaqMan® Fast Virus 1-Step Master Mix is an appropriate tool for performing the following types of experiments.

- **Presence/absence**
  - An endpoint experiment that indicates the presence or absence of a specific nucleic acid sequence (target) in a sample.
  - The actual quantity of target is not determined.
  - Presence/absence experiments are commonly used to detect the presence or absence of viral or bacterial pathogens. (Presence/absence experiments are also called *plus/minus experiments*.)
- **Standard curve**
  - A type of quantification experiment that determines the absolute target quantity in samples.
  - With the standard curve method, the real-time PCR system software measures amplification of the target in samples and in a standard dilution series.
  - Data from the standard dilution series are used to generate the standard curve.
  - Using the standard curve, the software interpolates the absolute quantity of target in the samples.

- Standard curve experiments are commonly used for quantifying viral load. (Standard curve experiments are also called *absolute quantification* or *AQ experiments*.)
- To collect only the  $C_t$  values, perform a standard curve experiment without running standards.
- **Relative quantification**
  - A type of quantification experiment that compares changes in gene expression in a given sample relative to another reference sample, such as an untreated control sample.
  - Relative quantification (RQ) can be performed with data from all real-time PCR instruments.
  - Relative quantification uses the standard curve and comparative  $C_t$  calculation methods.
  - Relative quantification does not allow single-sample results to be meaningful, nor does it allow gene-to-gene quantitative comparisons. It is used for sample-to-sample quantitative comparisons.

**Note:** A quantification experiment is a real-time experiment that measures the quantity of a target nucleic acid sequence (target) during each amplification cycle of the polymerase chain reaction (PCR).

## Determine the number of reactions

Determine the total number of reactions in your experiment. The following reaction types are required for each experiment type.

Experiment type	Reaction type	Description
Presence/ absence	Unknown	A well that can contain the following components: <ul style="list-style-type: none"> <li>• Sample (DNA or RNA in which the presence of a target is unknown)</li> <li>• TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix</li> <li>• Gene expression assay of choice</li> </ul>
	Exogenous Internal positive control (IPC)	A short synthetic DNA template that you can add to the PCRs to distinguish between true negative results and reactions affected by PCR inhibitors, incorrect assay setup, or a reagent or instrument failure. <b>Note:</b> We recommend the TaqMan <sup>®</sup> Exogenous Internal Positive Control Reagents Kit [Cat. No. 4308323].
	No amplification control (NAC)	A well that contains all reaction components, except the unknown sample and IPC. Alternatively, the well may contain the IPC plus a blocking agent for the IPC. No amplification should occur in NAC wells.
	No-template control (NTC)	A well that contains all PCR components, except the unknown sample. Only the IPC should amplify in NTC wells.
	Replicate	A reaction that is identical to another. It contains identical components and volumes. We recommend performing at least three replicates of each reaction.

Experiment type	Reaction type	Description
Standard curve	Unknown	A well that can contain the following components: <ul style="list-style-type: none"> <li>• Sample (DNA or RNA in which the quantity of the target is unknown)</li> <li>• TaqMan® Fast Virus 1-Step Master Mix</li> <li>• Gene expression assay of choice</li> </ul>
	Standard	A reaction that contains known standard quantities. It is used in quantification experiments to generate standard curves. <b>Note:</b> You can perform a standard curve experiment without running standards, if you only want to collect the $C_t$ values.
	Standard dilution series	A set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards.
	No template control (NTC)	A negative control well that contains water or buffer instead of sample. No amplification of the target should occur in negative control wells.
	Replicate	A reaction that is identical to another. It contains identical components and volumes. We recommend performing at least three replicates of each reaction.
Relative quantification	Experimental	A well that contains the experimental sample, Master Mix and assay, for the gene of interest or endo control.
	Reference	A reaction that contains the reference sample for the $\Delta\Delta C_t$ analysis, Master Mix and assay, for the gene of interest or endo control.
	No-template control (NTC)	A negative control well that contains water or buffer instead of sample. No amplification of the target should occur in negative control wells.
	Replicate	A reaction that is identical to another. It contains identical components and volumes. We recommend performing at least three replicates of each reaction.

## Determine optimal primer concentrations

With your custom-designed assay, determine the primer concentrations to use to obtain the earliest threshold cycle ( $C_t$ ) and the maximum baseline-corrected normalized reporter ( $\Delta R_n$ ).

**Note:** For information about how to select an amplicon site, design probes and primers, and calculate oligonucleotide concentrations, see [thermofisher.com/qpcducation](http://thermofisher.com/qpcducation).

Primer concentrations to test

Forward primer final concentration	Reverse primer final concentration	
	400 nM	900 nM
400 nM	400 nM/400 nM	400 nM/900 nM
900 nM	900 nM/400 nM	900 nM/900 nM

**Note:** These primer concentrations are for singleplex assays. For multiplex assays, see *TaqMan® Multiplex PCR Optimization User Guide* (Pub. No. MAN0010189).

## Prepare and run the RT-PCR reactions

Thaw the reagents and nucleic acid samples on ice. Resuspend the nucleic acid samples by inverting the tube, then gently vortexing.

1. Mix the TaqMan® Fast Virus 1-Step Master Mix thoroughly but gently until homogenous.
2. Prepare the RT-PCR Reaction Mix for the number of reactions required as shown in table below, plus 10% overage.

Component	Volume per well	
	384-well Plate or 96-well Fast (0.1-mL) Plate	96-well Standard (0.2-mL) Plate
TaqMan® Fast Virus 1-Step Master Mix	5 µL	12.5 µL
TaqMan® Gene Expression Assay or Custom TaqMan® Gene Expression Assay (20X) <sup>[1]</sup>	1 µL	2.5 µL
RT-PCR Grade Water	Varies <sup>[2]</sup>	Varies <sup>[3]</sup>
<b>Total RT-PCR Reaction Mix volume per reaction</b>	<b>20 µL</b>	<b>50 µL</b>

<sup>[1]</sup> If you are not using preformulated TaqMan® Gene Expression Assays, we recommend primer concentrations of 400–900 nM and a probe concentration of 100–250 nM.

<sup>[2]</sup> Sample volume will vary depending on experiment type. Add sufficient water to bring total to 20 µL *after* accounting for sample volume.

<sup>[3]</sup> Sample volume will vary depending on experiment type. Add sufficient water to bring total to 50 µL *after* accounting for sample volume.

3. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

---

**IMPORTANT!** The Master Mix is viscous because it is at 4X concentration. Ensure that all components are mixed thoroughly.

---

4. Transfer the RT-PCR Reaction Mix to the appropriate wells of a reaction plate.

**Note:** These volumes are recommended when working with viruses, as larger volumes are typically required to detect the low abundant virus. For targets present in high abundance, total volume can be decreased to 10 µL for the 384-well Plate or 96-well Fast (0.1-mL) Plate and 20 µL for the 96-well Standard (0.2-mL) Plate.

5. Add the following amounts of sample nucleic acid to the reaction plate wells.
  - 384-well Plate or 96-well Fast (0.1-mL) Plate: 1 pg to 100 ng
  - 96-well Standard (0.2-mL) Plate: 1 pg to 100 ng

**Note:** Do not use more than 1 µg of sample.

6. Seal the plate with an optical adhesive cover, then vortex briefly or invert the plate to mix the contents.

**Note:** Invert the plate for more uniform mixing because the Master Mix is viscous.

7. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

8. Select the appropriate cycling mode.  
TaqMan® Fast Virus 1-Step Master Mix is compatible with Fast or Standard cycling mode.

9. Set up the thermal protocol for your instrument.

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

**Table 5** Standard cycling mode (reaction volume >30 µL)

Step	Temperature	Time	Cycles
Reverse transcription <sup>[1]</sup>	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	15 seconds	40
Anneal / extend <sup>[2]</sup>	60°C	60 seconds	

<sup>[1]</sup> RT enzyme will function best in the range of 48–55°C.

<sup>[2]</sup> Ensure that the annealing temperature is consistent with the melting temperature ( $T_m$ ) of your primer designs.

**Table 6** Fast cycling mode (reaction volume <30 µL)

Step	Temperature	Time	Cycles
Reverse transcription <sup>[1]</sup>	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / extend <sup>[2]</sup>	60°C	30 seconds	

<sup>[1]</sup> RT enzyme will function best in the range of 48–55°C.

<sup>[2]</sup> Ensure that the annealing temperature is consistent with the melting temperature ( $T_m$ ) of your primer designs.

10. Set the reaction volume appropriate for the reaction plate.

- 96-well Standard (0.2-mL) Plate: **50 µL**
- 384-well Plate or 96-well Fast (0.1-mL) Plate: **20 µL**

11. Load the plate into the real-time PCR instrument.

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

12. Start the run.

**Analyze the results**

1. Review the  $\Delta R_n$  values to identify the optimal primer concentrations for PCR yield.
2. Review the  $C_t$  values to identify the optimal primer concentrations for  $C_t$  and detect any potential nonspecific amplification in the negative controls.
3. Select the forward primer and reverse primer combination that produces the earliest  $C_t$  and the highest  $\Delta R_n$ .

**Determine optimal probe concentration**

With your custom-designed assay, determine the probe concentration to use to obtain the earliest threshold cycle ( $C_t$ ) for the target sequence.

**Probe concentrations to test**

Use the TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix to prepare four replicate reactions with final probe concentrations of 100 nM and 250 nM. Use the optimal primer concentrations previously determined (“Prepare and run the RT-PCR reactions” on page 20).

**Prepare and run the RT-PCR reactions**

Thaw the reagents and nucleic acid samples on ice. Resuspend the nucleic acid samples by inverting the tube, then gently vortexing.

1. Mix the TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix thoroughly but gently until homogenous.
2. Prepare the RT-PCR Reaction Mix for the number of reactions required as shown in table below, plus 10% overage.

Component	Volume per well	
	384-well Plate or 96-well Fast (0.1-mL) Plate	96-well Standard (0.2-mL) Plate
TaqMan <sup>®</sup> Fast Virus 1-Step Master Mix	5 $\mu$ L	12.5 $\mu$ L
TaqMan <sup>®</sup> Gene Expression Assay or Custom TaqMan <sup>®</sup> Gene Expression Assay [20X] <sup>[1]</sup>	1 $\mu$ L	2.5 $\mu$ L
RT-PCR Grade Water	Varies <sup>[2]</sup>	Varies <sup>[3]</sup>
<b>Total RT-PCR Reaction Mix volume per reaction</b>	<b>20 <math>\mu</math>L</b>	<b>50 <math>\mu</math>L</b>

<sup>[1]</sup> If you are not using preformulated TaqMan<sup>®</sup> Gene Expression Assays, we recommend primer concentrations of 400–900 nM and a probe concentration of 100–250 nM.

<sup>[2]</sup> Sample volume will vary depending on experiment type. Add sufficient water to bring the total to 20  $\mu$ L *after* accounting for sample volume

<sup>[3]</sup> Sample volume will vary depending on experiment type. Add sufficient water to bring the total to 50  $\mu$ L *after* accounting for sample volume

3. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

---

**IMPORTANT!** The Master Mix is viscous because it is at 4X concentration. Ensure that all components are mixed thoroughly.

---

4. Transfer the RT-PCR Reaction Mix to the appropriate wells of a reaction plate.  
**Note:** These volumes are recommended when working with viruses, as larger volumes are typically required to detect the low abundant virus. For targets present in high abundance, total volume can be decreased to 10 µL for the 384-well Plate or 96-well Fast (0.1-mL) Plate and 20 µL for the 96-well Standard (0.2-mL) Plate.
5. Add the following amounts of sample nucleic acid to the reaction plate wells.
  - 384-well Plate or 96-well Fast (0.1-mL) Plate: 1 pg to 100 ng
  - 96-well Standard (0.2-mL) Plate: 1 pg to 100 ng**Note:** Do not use more than 1 µg of sample.
6. Seal the plate with an optical adhesive cover, then vortex briefly or invert the plate to mix the contents.  
**Note:** Invert the plate for more uniform mixing because the Master Mix is viscous.
7. Centrifuge the plate briefly to collect the contents at the bottom of the wells.
8. Select the appropriate cycling mode.  
TaqMan® Fast Virus 1-Step Master Mix is compatible with Fast or Standard cycling mode.
9. Set up the thermal protocol for your instrument.  
**Note:** The instrument must be configured with the appropriate block for the plate type.

**Table 7** Standard cycling mode (reaction volume >30 µL)

Step	Temperature	Time	Cycles
Reverse transcription <sup>[1]</sup>	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	15 seconds	40
Anneal / extend <sup>[2]</sup>	60°C	60 seconds	

<sup>[1]</sup> RT enzyme will function best in the range of 48–55°C.

<sup>[2]</sup> Ensure that the annealing temperature is consistent with the melting temperature ( $T_m$ ) of your primer designs.

**Table 8** Fast cycling mode (reaction volume <30  $\mu\text{L}$ )

Step	Temperature	Time	Cycles
Reverse transcription <sup>[1]</sup>	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / extend <sup>[2]</sup>	60°C	30 seconds	

<sup>[1]</sup> RT enzyme will function best in the range of 48–55°C.

<sup>[2]</sup> Ensure that the annealing temperature is consistent with the melting temperature ( $T_m$ ) of your primer designs.

10. Set the reaction volume appropriate for the reaction plate.

- 96-well Standard (0.2-mL) Plate: **50  $\mu\text{L}$**
- 384-well Plate or 96-well Fast (0.1-mL) Plate: **20  $\mu\text{L}$**

11. Load the plate into the real-time PCR instrument.

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

12. Start the run.

1. Review the  $\Delta R_n$  values to identify the optimal probe concentration for PCR yield.
2. Review the  $C_t$  values to identify the optimal probe concentration for  $C_t$  and detect any potential nonspecific amplification in the negative controls.
3. Select the probe concentration that produces the earliest  $C_t$  and the highest  $\Delta R_n$ .

## Analyze the results





# 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.

## 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/bmb15/BMBL.pdf](http://www.cdc.gov/biosafety/publications/bmb15/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](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)
-

# Documentation and support

## Related documentation

Document	Pub. No.
<i>TaqMan<sup>®</sup> Fast Virus 1-Step Master Mix Quick Reference</i>	4444464
<i>Introduction to Gene Expression Getting Started Guide</i>	4454239
<i>TaqMan<sup>®</sup> Gene Expression Assays User Guide—single-tube assays</i>	4333458
<i>Custom TaqMan<sup>®</sup> Assays Design and Ordering Guide</i>	4367671
<i>Primer Express<sup>™</sup> Software Version 3.0 Getting Started Guide</i>	4362460
<b>QuantStudio<sup>™</sup> 3 or 5 Real-Time PCR System</b>	
<i>QuantStudio<sup>™</sup> 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>QuantStudio<sup>™</sup> Design and Analysis Desktop Software User Guide</i>	MAN0010408
<b>QuantStudio<sup>™</sup> 6 / QuantStudio<sup>™</sup> 7 Flex Real-Time PCR System</b>	
<i>QuantStudio<sup>™</sup> 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i>	4489821
<i>QuantStudio<sup>™</sup> 6 and 7 Flex Real-Time PCR System Software Getting Started Guide</i>	4489822
<b>QuantStudio<sup>™</sup> 12K Flex Real-Time PCR System</b>	
<i>QuantStudio<sup>™</sup> 12K Flex Real-Time PCR System Maintenance and Administration Guide</i>	4470689
<i>QuantStudio<sup>™</sup> 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide</i>	4470050
<b>StepOne<sup>™</sup> or StepOnePlus<sup>™</sup> Real-Time PCR System</b>	
<i>StepOne<sup>™</sup> and StepOnePlus<sup>™</sup> Real-Time PCR Systems Installation, Networking and Maintenance User Guide</i>	4376782
<i>Applied Biosystems<sup>™</sup> StepOne<sup>™</sup> and StepOnePlus<sup>™</sup> Real-Time PCR Systems Relative Standard Curve and Comparative C<sub>t</sub> Experiments Getting Started Guide</i>	4376785
<b>ViiA<sup>™</sup> 7 Real-Time PCR System</b>	
<i>Applied Biosystems<sup>™</sup> ViiA<sup>™</sup> 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security</i>	4442661
<i>Applied Biosystems<sup>™</sup> ViiA<sup>™</sup> 7 Real-Time PCR System Getting Started Guide</i>	4441434

Document	Pub. No.
<b>7500/7500 Fast Real-Time PCR System</b>	
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</i>	4347828
<i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C<sub>t</sub> Experiments</i>	4387783
<i>Real-Time PCR Systems Chemistry Guide: Applied Biosystems™ 7900HT Fast Real-Time PCR System and 7300/7500 Real-Time PCR Systems</i>	4348358
<i>Applied Biosystems™ 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C<sub>T</sub> Getting Started Guide</i>	4364016
<i>Applied Biosystems™ 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i>	4364014
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve</i>	4347825
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Quantitation using Comparative C<sub>t</sub></i>	4347824
<i>Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C<sub>t</sub> Experiments Getting Started Guide</i>	4376785
<i>Applied Biosystems™ Relative Quantitation Analysis Module User Guide</i>	MAN0014820
<i>Applied Biosystems™ Standard Curve Analysis Module User Guide</i>	MAN0014819

## 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).

