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TaqMan™ Fast Virus 1-Step Master Mix

USER GUIDE

For one–step RT-PCR of viral nucleic acid

Catalog Numbers 4444432, 4444434, 4444436

Publication Number MAN0028278

Revision A.0

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0028278

Revision	Date	Description
A.0	7 September 2022	New document for the TaqMan™ Fast Virus 1-Step Master Mix. This document replaces Pub. No. 4453800, Rev. E, with the following changes: • Information was added about a master mix option without the ROX™ dye as a passive reference dye ("Product description" on page 5). • The list of compatible real-time PCR systems was updated. • THE RNA Storage Solution was added as an option to stabilize purified RNA. • A step was added to set the passive reference dye to **ROX** when setting up the real-time PCR instrument. • The list of related documentation was updated.

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

Contents

1 Product information

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.

It contains the following components:

- 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.

TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™) is also available. It does not contain a passive reference dye. This enables the measurement of a dye in the channel that would be used to measure the ROX™ dye as a passive reference dye. For more information, see *TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™) User Guide* (Pub. No. MAN0026486).

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**, 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).

Design custom assays

Use Primer Express™ Software to design custom assays (primer and probe sets) for use with the master mix.

Contents and storage

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

[1] See packaging for expiration date.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

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

Table 1 Recommended products for isolation of RNA or DNA *(continued)*

Item	Source
DNase I, Amplification Grade	18068015
TURBO™ DNase (2 U/µL)	AM2239
RNA*later*™-ICE Frozen Tissue Transition Solution	AM7030
RNA*later*™ Stabilization Solution	AM7020
TRI Reagent™ Solution	AM9738

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

Item	Source
Instrument, one of the following:	
QuantStudio™ 6 Pro Real-Time PCR System or QuantStudio™ 7 Pro Real-Time PCR System	
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	
7900HT Fast Real-Time PCR System / 7900HT Real-Time PCR Instrument	
ViiA™ 7 Real-Time PCR System	
Or use a compatible real–time PCR instrument from another supplier. **Note:** Verify thermal cycling conditions on other real–time PCR instruments.	
Software	
Primer Express™ Software	4363991
Equipment	
Centrifuge with plate adapter	MLS
Microcentrifuge	MLS
Single- or multi-channel pipettes (electronic or manual)	MLS
Laboratory mixer (vortex or equivalent)	MLS

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

Item	Source
Tubes, plates, and other consumables	
Tubes, plates and film	**thermofisher.com/plastics**
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
TE, pH 8.0, RNase-free	AM9849
TaqMan™ Gene Expression Assays	**thermofisher.com/taqmangeneexpression**
Nuclease-Free Water (not DEPC-Treated)	AM9930
TaqMan™ Exogenous Internal Positive Control Reagents Kit	4308323
RT-PCR Grade Water	AM9935
Optional reagents to preserve or stabilize RNA	
RNA*later*™-ICE Frozen Tissue Transition Solution	AM7030
RNA*later*™ Stabilization Solution	AM7020
THE RNA Storage Solution	AM7000

Workflow

Workflow

Start with RNA, DNA, or RNA and DNA

See Chapter 2, “Guidelines for preparation of nucleic acid”.

Before you begin (60X assays) (page 12)

Prepare the RT–PCR Reaction Mix (page 12)

Set up and run the real–time PCR instrument (page 14)

Analyze the results (page 15)

2 Guidelines for preparation of nucleic acid

Starting template

RT–PCR with TaqMan™ 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

- For recommended kits to isolate RNA or DNA, see “Required materials not supplied” on page 6.
- Store isolated nucleic acid at –86°C to –10°C.
- The viral RNA isolation kits include carrier RNA to maximize RNA recovery.
- *(Optional)* Use RNA*later*™-ICE Frozen Tissue Transition Solution when thawing frozen tissue for RNA extraction to preserve the RNA.
- *(Optional)* Use RNA*later*™ Stabilization Solution to stabilize RNA in tissue.
- *(Optional)* Use THE RNA Storage Solution to stabilize purified RNA.

3 Prepare and run the RT-PCR reactions

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 the master mix on ice, then mix thoroughly but gently.

  Note: The master mix does not freeze at –25°C to –15°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.
- The master mix is designed to accommodate multiple assays. For guidelines on designing multiplex reactions, see *TaqMan™ Assay Multiplex PCR Optimization Application Guide* (Pub. No. MAN0010189).
- Verify the assays and optimize the thermal–cycling conditions for assays other than TaqMan™ Gene Expression Assays or Custom TaqMan™ Gene Expression Assays, or when using thermal–cycling conditions other than those specified in this protocol.

Note: (*Optional*) The master mix and the TaqMan™ Gene Expression Assay can be combined ahead of time and stored at –30°C to –10°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, RNase-free, 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 more information about the number of reactions, 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, RNase-free (1X)	80 µL
Total aliquot volume	**120 µL**

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 master mix thoroughly but gently until it is 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 (0.1-mL) plate	96-well (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)[1]	1 µL	2.5 µL
RT-PCR Grade Water	Varies[2]	Varies[3]
Total RT–PCR Reaction Mix volume per reaction	**20 µL**	**50 µL**

[1] 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.

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

[3] 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.
   - 20 µL for the 96-well (0.2-mL) plate
   - 10 µL for the 96-well (0.1-mL) plate or the 384-well plate

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

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.

   The 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[1]	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	15 seconds	40
Anneal / extend[2]	60°C	60 seconds	

[1] RT enzyme will function best in the range of 48–55°C.
[2] 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[1]	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / extend[2]	60°C	30 seconds	

[1] RT enzyme will function best in the range of 48–55°C.
[2] 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 (0.2-mL) plate: **50 µL**
   - 384–well plate or 96-well (0.1-mL) plate: **20 µL**
4. Set the passive reference dye to **ROX**.
5. Load the plate into the real–time PCR instrument.
6. 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 (C_q) 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/qpcreducation**.

A Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For troubleshooting information and FAQs for this product: **thermofisher.com/taqmanfastvirusmmfaqs**
- To browse the database and search using keywords: **thermofisher.com/faqs**

B

Supplemental information

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: • Sample (DNA or RNA in which the presence of a target is unknown) • Master mix • Gene expression assay of choice
	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. **Note:** We recommend the TaqMan™ 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.
Standard curve	Unknown	A well that can contain the following components: • Sample (DNA or RNA in which the quantity of the target is unknown) • Master mix • Gene expression assay of choice
	Standard	A reaction that contains known standard quantities. It is used in quantification experiments to generate standard curves. **Note:** You can perform a standard curve experiment without running standards, if you only want to collect the C_t (C_q) 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.

(continued)

Experiment type	Reaction type	Description
Standard curve	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 or C_q) and the maximum baseline-corrected normalized reporter (ΔR_n).

Note: For information about how to select an amplicon site, design probes and primers, and calculate oligonucleotide concentrations, see **thermofisher.com/qpcreducation**.

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™ Assay Multiplex PCR Optimization Application 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 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 (0.1-mL) plate	96-well (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)[1]	1 µL	2.5 µL
RT-PCR Grade Water	Varies[2]	Varies[3]
Total RT–PCR Reaction Mix volume per reaction	**20 µL**	**50 µL**

[1] 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.

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

[3] 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.

- 20 µL for the 96-well (0.2-mL) plate
- 10 µL for the 96-well (0.1-mL) plate or the 384-well plate

5. Add the following amounts of sample nucleic acid to the reaction plate wells.
   - 384–well Plate or 96-well (0.1-mL) plate: 1 pg to 100 ng
   - 96-well (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.
   The 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[1]	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	15 seconds	40
Anneal / extend[2]	60°C	60 seconds	

[1] RT enzyme will function best in the range of 48–55°C.
[2] 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[1]	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / extend[2]	60°C	30 seconds	

[1] RT enzyme will function best in the range of 48–55°C.
[2] 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 (0.2-mL) plate: **50 µL**
    - 384–well plate or 96-well (0.1-mL) plate: **20 µL**

11. Set the passive reference dye to **ROX**.

12. Load the plate into the real–time PCR instrument.

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

13. Start the run.

Analyze the results

1. Review the ΔR_n values to identify the optimal primer concentrations for PCR yield.
2. Review the C_t (C_q) values to identify the optimal primer concentrations for C_t (C_q) 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 (C_q) and the highest ΔR_n.

Determine optimal probe concentration

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

Probe concentrations to test

Use the master mix to prepare four replicate reactions with final probe concentrations of 100 nM and 250 nM. Use the optimal primer concentrations previously determined (see "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 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 (0.1-mL) plate	96-well (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)[1]	1 µL	2.5 µL
RT-PCR Grade Water	Varies[2]	Varies[3]
Total RT–PCR Reaction Mix volume per reaction	**20 µL**	**50 µL**

[1] 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.

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

[3] Sample volume will vary depending on experiment type. Add sufficient water to bring the 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.
   - 20 µL for the 96-well (0.2-mL) plate
   - 10 µL for the 96-well (0.1-mL) plate or the 384-well plate

5. Add the following amounts of sample nucleic acid to the reaction plate wells.
   - 384–well Plate or 96-well (0.1-mL) plate: 1 pg to 100 ng
   - 96-well (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.
   The 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[1]	50°C	5 minutes	1
RT inactivation / initial denaturation	95°C	20 seconds	1
Denature	95°C	15 seconds	40
Anneal / extend[2]	60°C	60 seconds	

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

[2] 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 µL)

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

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

[2] 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 (0.2-mL) plate: **50 µL**
    - 384–well plate or 96-well (0.1-mL) plate: **20 µL**
11. Set the passive reference dye to **ROX**.
12. Load the plate into the real–time PCR instrument.

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

Analyze the results

1. Review the ΔR_n values to identify the optimal probe concentration for PCR yield.
2. Review the C_t (C_q) values to identify the optimal probe concentration for C_t (C_q) and detect any potential nonspecific amplification in the negative controls.
3. Select the probe concentration that produces the earliest C_t (C_q) and the highest ΔR_n.

C 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.

D

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, visit **thermofisher.com/support**.

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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

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 **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) **www.who.int/publications/i/item/9789240011311**

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Documentation and support

Related documentation

Document	Pub. No.
TaqMan™ Fast Virus 1-Step Master Mix Quick Reference	4444464
TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™) User Guide	MAN0026486
TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™) Quick Reference	MAN0026487
Introduction to Gene Expression Getting Started Guide	4454239
TaqMan™ Gene Expression Assays User Guide—single-tube assays	4333458
Custom TaqMan™ Assays Design and Ordering Guide	4367671
Primer Express™ Software Version 3.0 Getting Started Guide	4362460
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Design and Analysis Desktop Software User Guide	MAN0010408
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.3) Maintenance and Administration Guide	4489821
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide	MAN0018828
QuantStudio™ Real-Time PCR Software Getting Started Guide	4489822
QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System	
QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System User Guide	MAN0018045
QuantStudio™ Design and Analysis Software v2 User Guide	MAN0018200
QuantStudio™ 12K Flex Real–Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide	MAN0018832
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050

(continued)

Document	Pub. No.
StepOne™ or StepOnePlus™ Real-Time PCR System	
StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide	4376782
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785
ViiA™ 7 Real-Time PCR System	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security	4442661
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide	4441434
7500/7500 Fast Real-Time PCR System / 7900HT Fast Real-Time PCR System	
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments	4387783
Real-Time PCR Systems Chemistry Guide: Applied Biosystems™ 7900HT Fast Real-Time PCR System and 7300/7500 Real-Time PCR Systems	4348358
Applied Biosystems™ 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide	4364016
Applied Biosystems™ 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide	4364014
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve	4347825
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Quantitation using Comparative C_t	4347824
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785
Applied Biosystems™ Relative Quantitation Analysis Module User Guide	MAN0014820
Applied Biosystems™ Standard Curve Analysis Module User Guide	MAN0014819

Customer and technical support

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

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

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

Limited product warranty

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