applied biosystems

TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™)

USER GUIDE

For one–step RT-PCR of viral nucleic acid

Catalog Numbers 5555532, 5555534, 5555536

Publication Number MAN0026486

Revision B

ThermoFisher SCIENTIFIC

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.

Revision history: MAN0026486 B (English)

Revision	Date	Description
B	9 March 2026	• The information about how to order TaqMan™ Gene Expression Assays was updated ("Order TaqMan™ Gene Expression Assays" on page 6). • Information was added about using the Custom TaqMan™ Assay Design Tool to design custom assays and enter primer and probe sequences ("Overview of custom assays" on page 6). • The Bio-Rad™ CFX Opus 96 Real-Time PCR System and Roche™ LightCycler™ PRO System were added ("Required materials not supplied" on page 6). • Information about dye compatibility on Bio-Rad™ and Roche™ real-time PCR systems was added ("Dye compatibility on third-party real-time PCR instruments" on page 12). • Thermal cycling guidance and a list of compatible run modes for supported real-time PCR systems were added ("Thermal cycling conditions for your real-time PCR system" on page 12). • Clarified that direct comparison of absolute C_t (C_q) values across different real-time PCR platforms is not recommended ("Analyze the results" on page 16). • Information was added about the items to review when analyzing standard curve experiments ("Analyze the results" on page 16). • The troubleshooting and FAQs section was updated (Appendix A, "Troubleshooting and FAQs"). • Clarification was added about experiment type variability ("Determine the number of reactions" on page 19). • Dye-selection guidance for multiplex reactions was added ("Dye selection" on page 25). • The related documentation section was updated ("Related documentation" on page 30). • Minor updates were made for consistency of style and terminology.
A.0	19 July 2022	New document for the TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™).

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 Multiplex Master Mix (No ROX™) 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
- Optimized buffer components

The TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™) enables one-step RT-PCR for presence/absence, standard curve, and relative quantification experiments. For information about the types of experiments, see "Experiment types" on page 18.

This master mix 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.

The TaqMan™ Fast Virus 1-Step Master Mix is available. It contains ROX™ dye as a passive reference dye. For more information, see *TaqMan™ Fast Virus 1-Step Master Mix User Guide* (Pub. No. MAN0028278).

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 explore our offerings and order pre-designed TaqMan™ Assays, use our TaqMan™ Assay Search Wizard (**https://www.thermofisher.com/order/taqman-search-wizard/#!/**).

To customize your own TaqMan™ Assay, go to our Assay Design Hub (**https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/assay-design.html**).

To order individual oligos, go to our Custom TaqMan™ Probe webpage (**https://www.thermofisher.com/order/custom-oligo/custom-taqman-probes**).

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

Overview of custom assays

The Custom TaqMan™ Assay Design Tool can be used to design custom assays. It can also be used to enter primer and probe sequences.

We recommend the Primer Express™ Software when entering primer and probe sequences in the Custom TaqMan™ Assay Design Tool.

Contents and storage

Cat. No.[1]	Contents	Number of 20–µL reactions	Storage[2]
5555532	1 × 1 mL	200	–25°C to –15°C
5555534	5 × 1 mL	1,000	
5555536	1 × 10 mL	2,000	

[1] Catalog numbers that appear as links open the web pages for those products.
[2] 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.

For more information about RNA or DNA isolation kits, go to **thermofisher.com/dnaextraction**.

Table 1 Recommended products for isolation of RNA or DNA

Item	Source
Isolation kits	
MagMAX™ CORE Nucleic Acid Purification Kit	A32702
MagMAX™-96 Viral RNA Isolation Kit	AM1836
MagMAX™-96 Total RNA Isolation Kit	AM1830

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

Item	Source
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
Other products	
TURBO DNA-*free*™ Kit	AM1907
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
THE RNA Storage Solution	AM7001

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

Item	Source
Real-time PCR instrument, one of the following:	
QuantStudio™ 3 or 5 Real-Time PCR Instrument	Contact your local sales office.
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 Pro Real-Time PCR System or QuantStudio™ 7 Pro 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 Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
CFX Opus 96 Real-Time PCR System	Bio-Rad™ 12011319
LightCycler™ PRO System	Roche™ 09541713001

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

Item	Source
Real-time PCR instruments from other manufacturers may also be compatible; optimize thermal cycling conditions as needed.[1]	—
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**
Tubes, plates, and other consumables	
Tubes, plates and film[2]	**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

[1] Contact Technical Support for guidance with instrument compatibility or optimization (see Appendix E, "Documentation and support").
[2] Confirm that all plastic consumables are compatible with the applicable real-time PCR system before use.

Workflow

Workflow

Start with RNA, DNA, or RNA and DNA

See "Guidelines for preparation of high-quality nucleic acid samples" on page 10.

Before you begin (60X assays) (page 13)

Prepare the RT–PCR reaction mix (page 13)

Set up and run the real–time PCR instrument (page 15)

Analyze the data (page 16)

2 Guidelines for preparation of nucleic acid

Starting template

RT–PCR 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 Table 1 on page 6.
- Store isolated nucleic acid at –86°C to –10°C.
- The recommended viral RNA isolation kits include carrier RNA to help 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

General guidelines

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.

Dye compatibility on third-party real-time PCR instruments

Dye compatibility varies among third-party real-time PCR instruments. Select dyes that are compatible with the instrument and with other dyes in the multiplex reaction.

ABY™ dye is not compatible with the Bio-Rad™ CFX Opus 96 Real-Time PCR System or Roche™ LightCycler™ PRO System. The emission spectrum of ABY™ dye overlaps 2 optical detection channels on these instruments. Use Cyanine 5 dye instead.

For instrument-specific information about dye compatibility, see the user guide for your instrument. For general guidance on reporter dye selection in multiplex assays, see “Dye selection” on page 25.

Contact Technical Support for questions about dye compatibility or instrument setup.

Thermal cycling conditions for your real-time PCR system

The master mix can be run in either Fast or Standard run modes, depending on the real-time PCR system. Select a run mode supported by your instrument that aligns with the specified thermal cycling profile.

- **Thermal cycling profile—**The thermal cycling profile defines the temperature and time for each step. Use the appropriate thermal cycling profile for your system.
- **Run mode—**The run mode defines the ramp rate that is used to heat or cool the sample block between temperature changes.

Product performance was evaluated using the run modes listed in the following table.

Real-time PCR system[1]	Compatible run modes
QuantStudio™ 3 and 5 Real-Time PCR Systems	Standard/Fast
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems	Standard/Fast
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	Standard/Fast
QuantStudio™ 12K Flex Real–Time PCR System	Standard/Fast
ViiA™ 7 Real-Time PCR System	Standard/Fast
StepOnePlus™ Real-Time PCR System	Standard/Fast
StepOne™ Real-Time PCR System	Standard
7500 Fast Real-Time PCR System	Standard/Fast
7500 Real-Time PCR System	Standard
7900HT Real-Time PCR System	Standard/Fast
Bio-Rad™ CFX Opus 96 Real-Time PCR System	Fast
Roche™ LightCycler™ PRO System	Fast

[1] For more information on available real-time PCR systems, accessories and consumables, go to thermofisher.com.

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 19.

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 homogeneous.

2. Prepare the RT–PCR reaction mix according to the following table for the required number of reactions, plus 10% overage.

Component	Volume per reaction	
	384–well plate or 96-well (0.1-mL) plate	96-well (0.2-mL) plate
TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™)	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	**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 varies by experiment type. Add water to reach a total volume of 20 µL, including the sample.

[3] Sample volume varies by experiment type. Add water to reach a total volume of 50 µL, including the sample.

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 due to its 4X formulation and must be 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.
   - 96-well (0.2-mL) plate: 1 pg to 100 ng
   - 384–well plate or 96-well (0.1-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] The RT enzyme performs best between 48–55°C.

[2] Confirm 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] The RT enzyme performs best between 48–55°C.

[2] Confirm 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. Select **None** for the passive reference dye if applicable to the instrument.
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.

Note: Analyze data using the native software for each real-time PCR instrument. Do not directly compare absolute C_t (C_q) values between Applied Biosystems™ instruments and the Bio-Rad™ CFX Opus 96 Real-Time PCR System or Roche™ LightCycler™ PRO System. Trends and relative differences should be consistent; however, absolute values can differ due to instrument-specific data-analysis algorithms.

The real-time PCR system software can be used to set the baseline and threshold values for the amplification plot. They can be set automatically or manually.

The baseline is the initial cycle in which there is a change in the fluorescence signal.

The intersection of the threshold and the amplification plot defines the C_t value. In real-time PCR assays, the threshold is set above the background signal and within the exponential growth phase of the amplification curve.

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 standard curve experiments, view the following items:

- Slope
- Amplification efficiency
- R^2 value
- Y-intercept
- C_t values
- Outliers

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

For troubleshooting information and FAQs for this product, go to: **thermofisher.com/taqman-fast-virus-no-rox-mm-faqs**

B

Supplemental information

Experiment types

The master mix is compatible with 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 (C_q) 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[1]	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[1]	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.

[1] The experiment type, required controls, and supported results can vary depending on the real-time PCR instrument, software, and vendor.

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 homogeneous.

2. Prepare the RT–PCR reaction mix according to the following table for the required number of reactions, plus 10% overage.

Component	Volume per reaction	
	384–well plate or 96-well (0.1-mL) plate	96-well (0.2-mL) plate
TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™)	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	**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 varies by experiment type. Add water to reach a total volume of 20 µL, including the sample.

[3] Sample volume varies by experiment type. Add water to reach a total volume of 50 µL, including the sample.

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 due to its 4X formulation and must be 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.
   - 96-well (0.2-mL) plate: 1 pg to 100 ng
   - 384–well Plate or 96-well (0.1-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] The RT enzyme performs best between 48–55°C.
[2] Confirm 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] The RT enzyme performs best between 48–55°C.
[2] Confirm 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. Select **None** for the passive reference dye if applicable to the instrument.

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 21).

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 homogeneous.
2. Prepare the RT–PCR reaction mix for the required number of reactions according to the following table, plus 10% overage.

Component	Volume per reaction	
	384–well plate or 96-well (0.1-mL) plate	96-well (0.2-mL) plate
TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™)	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	**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 varies by experiment type. Add water to reach a total volume of 20 µL, including the sample.
[3] Sample volume varies by experiment type. Add water to reach a total volume of 50 µL, including the sample.

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 due to its 4X formulation and must be 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.
   - 96-well (0.2-mL) plate: 1 pg to 100 ng
   - 384–well Plate or 96-well (0.1-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] The RT enzyme performs best between 48–55°C.

[2] Confirm 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] The RT enzyme performs best between 48–55°C.

[2] Confirm 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. Select **None** for the passive reference dye if applicable to the instrument.
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.

Dye selection

It is recommended to make dye/target assignments to balance fluorescence levels in the multiplex reaction.

- FAM™ and ABY™ dyes used with low to medium expressors.
- VIC™ and JUN™ dyes used with medium to high expressors.

Note: Some reporter dyes might not be compatible with certain third-party instruments. For more information, see “Dye compatibility on third-party real-time PCR instruments” on page 12.

C Suggested 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.

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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

E

Documentation and support

Related documentation

Document	Pub. No.
TaqMan™ Fast Virus 1-Step Multiplex Master Mix (No ROX™) Quick Reference	MAN0026487
TaqMan™ Fast Virus 1-Step Master Mix User Guide	MAN0028278
TaqMan™ Fast Virus 1-Step Master Mix Product Information Sheet	4444464
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 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™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide	MAN0018828
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.3) Maintenance and Administration Guide	4489821
QuantStudio™ Real-Time PCR Software Getting Started Guide	4489822
QuantStudio™ 12K Flex Real–Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide	MAN0018832
QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide	4470689
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	
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 its affiliates 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 questions, contact Life Technologies at **www.thermofisher.com/support**.