TaqMan® Fast Virus 1-Step Master Mix

Catalog Numbers 4444432, 4444434, 4444436

Pub. No. 4444464 **Rev.** B

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Fast Virus 1-Step Master Mix User Guide* (Pub. No. 4453800). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of TaqMan® Fast Virus 1-Step Master Mix. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® Fast Virus 1-Step Master Mix User Guide* (Pub. No. 4453800).

Product description

The TaqMan® Fast Virus 1-Step Master Mix can be used with any TaqMan® primer and probe set for real-time RT-PCR of DNA and RNA samples. During thermal cycling, the reverse transcription step does not affect performance with DNA targets.

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.

Perform RT-PCR

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.

Component	Volume
TaqMan® Gene Expression Assays (60X) or Custom TaqMan® Gene Expression Assays (60X)	40 µL
TE, pH 8.0 (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 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)[1]	1 µL	2.5 µL
RT-PCR Grade Water	14 µL	35 µL
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.

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.

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.

   Note: The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does *not* depend on a Standard or Fast plate format.

2. Set up the thermal protocol.

Table 1 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	40

[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 2 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	40

[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 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/qpcreducation**.

Limited product warranty

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Revision	Date	Description
B	19 September 2018	Updated for manufacturer, general style, formatting, and branding.
A	May 2010	Baseline for this revision history.