TaqMan® Gene Expression Assays—single-tube assays

Pub. No. 4401212 **Rev.** D

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458). 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® Gene Expression Assays—single-tube assays. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458).

Procedural guidelines

Guidelines for preparing cDNA templates

- For optimal reverse transcription, input RNA should be:
  - Free of inhibitors of reverse transcription (RT) and PCR
  - Dissolved in PCR-compatible buffer
  - Free of RNase activity

    Note: We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA
- For the input RNA amount, follow the recommendations provided by the cDNA kit.
- Small amounts of cDNA can be pre-amplified. Use TaqMan® PreAmp Master Mix (Cat. No. 4391128) or TaqMan® PreAmp Master Mix Kit (Cat. No. 4384267).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.

Procedural guidelines for performing real-time PCR

- 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.
- Run technical replicates in triplicate to identify outliers.

Perform PCR amplification

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 PCR Reaction Mix

Thaw the cDNA samples on ice. Resuspend the cDNA samples by inverting the tube, then gently vortexing.

1. Mix the Master Mix thoroughly but gently.
2. Combine the PCR Reaction Mix and assays in an appropriately-sized microcentrifuge tube according to the following table.

Component	Volume for 1 reaction	
	Standard 96-well or 48-well Plates	**384-well Plate or 96-well Fast Plate**
Master Mix (2X)[1,2]	10 µL	5 µL
TaqMan® Gene Expression Assay (20X) or Custom TaqMan® Gene Expression Assay (20X)	1 µL	0.5 µL
Nuclease-free water[3]	7 µL	3.5 µL
Total PCR Reaction Mix volume	**18 µL**	**9 µL**

[1] Recommended: TaqMan® Fast Advanced Master Mix

[2] (*Optional*) If you add AmpErase™ UNG (uracil-N-glycosylase), the final concentration must be 0.01U/ µL. Reduce the volume of water in the PCR reaction mix to compensate for additional volume from the UNG.

[3] Adjust the volume of nuclease-free water for a larger volume of cDNA.

3. Vortex the PCR Reaction Mix, then centrifuge briefly.
4. Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.

5. Add cDNA template (1 pg–100 ng in nuclease-free water), or nuclease-free water for NTC, to each well.
   - 1 µL for a 384-well plate or 96-well Fast Plate
   - 2 µL for a 96-well and 48-well Standard Plate

   Note: Be sure to adjust the volume of nuclease-free water in the PCR reaction mix for a larger volume of cDNA.

   IMPORTANT! For optimal results when using TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG, prepare the plate on ice. Run the plate within 2 hours of preparation, or store the plate at 2–8°C for up to 24 hours.

6. Seal the plate with a MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.

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 user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

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

1. Select the cycling mode appropriate for the Master Mix.

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

2. Set up the thermal protocol for your instrument.

   See "Thermal protocols" on page 2 for the thermal protocols for other Master Mixes.

   Table 1 TaqMan® Fast Advanced Master Mix (StepOne™, StepOnePlus™, ViiA™ 7, and QuantStudio™ systems with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

[1] Optional, for optimal UNG activity.
[2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time.

   Table 2 TaqMan® Fast Advanced Master Mix (7500 and 7500 Fast systems with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

[1] Optional, for optimal UNG activity.
[2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time

3. Set the reaction volume appropriate for the reaction plate.
   - **96-well Standard (0.2-mL) Plate**: 20 µL
   - **96-well Fast (0.1-mL) Plate and 384–well Plate**: 10 µL

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

5. Start the run.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your instrument. Use the absolute or relative quantification ($\Delta\Delta C_t$) methods to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values.
  - Remove outliers from the analysis.
- 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 **thermofisher.com/qpcreducation**.

Thermal protocols

The thermal protocols in "Set up and run the real-time PCR instrument" on page 2 are optimized for the TaqMan® Fast Advanced Master Mix.

The following tables provide thermal protocols for other Master Mixes that are compatible with TaqMan® Gene Expression Assays.

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

Table 3 TaqMan® Gene Expression Master Mix or TaqMan® Universal Master Mix II, with UNG (any compatible instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	1 minute	

[1] For optimal UNG activity.

Table 4 TaqMan® Universal Master Mix II, no UNG (any compatible instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	1 minute	

Table 5 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (StepOne™, StepOnePlus™, ViiA™ 7, or QuantStudio™ system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

Table 6 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (7500 or 7500 Fast system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

Enzyme activation time

Using TaqMan® Fast Advanced Master Mix, the enzyme activation step can range from 20 seconds to 2 minutes. A 20–second enzyme activation step is sufficient when the template is cDNA. A longer enzyme activation time should not cause different results. The enzyme activation time for the default fast thermal cycling conditions on the instruments is 20 seconds. If a longer enzyme activation time is required, the thermal cycling conditions need to be changed before the run is started. A longer enzyme activation time can help to denature double-stranded genomic DNA when genomic DNA is used.

Limited product warranty

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Revision	Date	Description
D	15 May 2018	• Added procedural guidelines. • Added new instruments, Master Mixes, and other products applicable for the workflows. • Updated for general style, formatting, and branding.
C	November 2010	Baseline for this revision history.