TaqMan® Gene Expression Assays —96-well Standard (0.2-mL) TaqMan® Array Plates

Pub. No. 4391139 **Rev.** E

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Plates* (Pub. No. 4391016). 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 and 96-well Standard (0.2-mL) TaqMan® Array Plates. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Plates* (Pub. No. 4391016).

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

- Keep the plate protected from light and stored as indicated until ready for use.
- Use the same quantity of cDNA sample for all reactions. We recommended using 1 to 100 ng of cDNA per well for 96-well Standard Plates.

Perform PCR amplification

Combine cDNA and Master 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 cDNA and Master Mix in an appropriately-sized microcentrifuge tube.

   Table 1 96-well Standard (0.2-mL) Plate

Component	Volume			
	1 well	8 wells [1]	32 wells [1]	96 wells [1]
cDNA sample + nuclease-free water[2]	10 µL	90 µL	360 µL	1,080 µL
Master Mix (2X)[3]	10 µL	90 µL	360 µL	1,080 µL
Total volume	**20 µL**	**180 µL**	**720 µL**	**2,160 µL**

   [1] Includes 12.5% overage.
   [2] Ensure that the final cDNA concentration per well is 1–100 ng per 20-µL reaction.
   [3] TaqMan® Fast Advanced Master Mix is recommended.

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

Prepare the TaqMan® Array Plate

1. Remove the plate from its packaging, centrifuge briefly, then remove the plate cover.
2. Add the cDNA-Master Mix to the appropriate wells of the plate.

   Use 20 µL per well.

   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.
3. Seal the plate with MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.
4. Centrifuge the plate briefly to collect the contents to the bottom of the wells.

Set up and run the real-time PCR instrument

1. Import the setup file (SDS in TXT format) into the real-time PCR instrument or software.

2. 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.

3. Set up the thermal protocol for your instrument.

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

   Table 2 TaqMan® Fast Advanced Master Mix (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 3 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.

4. 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**: 10 µL

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

6. Start the run.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your instrument.

Use 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.
  - 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.

Perform additional data analysis using any of the following software:

Software	Resource
Relative Quantification app	**thermofisher.com/cloud**
ExpressionSuite™ Software[1]	**thermofisher.com/expressionsuite**

[1] Can automatically define the baseline.

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

Data can be analyzed using the relative threshold algorithm (C_{rt}).

Use the relative threshold algorithm in your software. If your software does not have the relative threshold algorithm, you can use the Relative Quantification app that is available on the Thermo Fisher Cloud.

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 4 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 5 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 6 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (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 7 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
E	15 May 2018	• Added new instruments, Master Mixes, and other applicable products. • Added thermal cycling protocols for all compatible Master Mixes. • Added procedural guidelines. • Updated for general style, formatting, and branding.
D	September 2011	Baseline for this revision history.