TaqMan® Pri-miRNA Assays

Pub. No. 4427720 **Rev.** C

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *TaqMan® Pri-miRNA Assays User Guide* (Pub. No. 4427719). 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® Pri-miRNA Assays. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® Pri-miRNA Assays User Guide* (Pub. No. 4427719).

Procedural guidelines

Guidelines for cDNA template preparation

- Use the same reverse transcription procedure for all samples.
- 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
  - Nondegraded total RNA (not applicable for double-stranded templates)

  IMPORTANT! Degradation of the RNA can reduce the yield of cDNA for some gene targets.
- For the input RNA amount, follow the recommendations that are provided by the cDNA kit.
- Denaturation of the RNA is not necessary and can reduce the yield of cDNA for some gene targets.
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.
- If using strip tubes to prepare cDNA templates, change to a new cap after each step or incubation.
- cDNA samples that will not be immediately processed can be stored at −25°C to −15°C.
- Quantify cDNA by with the TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831) or UV absorbance (A_{260}/A_{280}).
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes to prepare cDNA templates.

Procedural guidelines for performing real-time PCR

- Use the same amount of cDNA in each reaction.
- Prepare the real-time PCR reactions in an area free of artificial templates and siRNA transfections. High-copy-number templates can easily contaminate the real-time PCR reactions.
- Configure run documents according to the instructions provided in the real-time PCR instrument user documents.
- 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 four technical replicates to identify outliers.

Perform PCR amplification

Before you begin

- Determine the total number of PCR reactions needed, including replicates, for each sample. Include an endogenous control and a no template control for each assay.
- Thaw the assays on ice, then vortex and briefly centrifuge to resuspend.
- Dilute 60X assays to 20X working stocks with TE, pH 8.0. Then, divide into smaller aliquots to minimize freeze-thaw cycles.

  Note: The size of the aliquots depends on the number of planned PCR reactions.

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® Pri-miRNA Assay (20X)	1 µL	0.5 µL
Nuclease-free Water[3]	5 µL	2.5 µL
PCR Reaction Mix volume	**16 µL**	**8 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 to for a larger volume of cDNA

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

4. Transfer the appropriate volume of PCR Reaction Mix to each well of an optitcal reaction plate.

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

   Note: 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 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 3 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	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

[1] Optional, for optimal UNG activity.

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	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

[1] Optional, for optimal UNG activity.

3. Set the reaction volume appropriate for the reaction plate.
   - 96 or 48-well Standard (0.2-mL) plates: **20 µL**
   - 96-well Fast (0.1-mL) Plate and 384–well plates: **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**.

Algorithms for data analysis

Table 3 Algorithm recommendations for single–tube assays

Algorithm	Recommendation
Threshold (C_t)	Recommended.
Relative threshold (C_{rt})	*(Optional)* Use for troubleshooting abnormal or unexpected results.

The relative threshold algorithm is available in the Relative Quantification application on Connect (**thermofisher.com/connect**).

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® Pri-miRNA 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 (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 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	

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Revision	Date	Description
C	16 July 2019	• Added procedural guidelines. • Added new instruments, Master Mixes, and other applicable products. • Updated the order that components are added to the PCR Reaction Mix. • Updated for general style, formatting, and branding.
B	May 2009	Baseline for this revision history.

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