TaqMan® Gene Expression Assays—TaqMan® Array Cards

Pub. No. 4371129 **Rev.** C

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Cards* (Pub. No. 4400263). 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 TaqMan® Array Cards. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Cards* (Pub. No. 4400263).

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

- Before preparing a TaqMan® Array Card, review *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Cards* (Pub. No. 4400263).
- 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.
- Keep the card protected from light and stored as indicated until ready for use. Excessive exposure to light may affect the fluorescent probes of the dried-down assays in the card.
- Use the same quantity of cDNA sample for all reactions. Use a cDNA quantity of 30–1,000 ng per fill reservoir (0.3–10 ng/µL).
- Load each fill reservoir with 100 µL of sample-specific PCR reaction mix.
  - Each fill reservoir contains a single sample as determined by the card layout.
  - The 100–µL volume ensures adequate filling of each reaction well. Volumes smaller than 100 µL result in insufficiently filled cards.
- Equilibrate the card that is loaded with PCR reaction mix to room temperature before loading into the real-time PCR instrument.
- If the card is not run immediately, protect it from light and store at 2-8°C.

Perform real-time PCR

Combine cDNA and Master Mix

Note: Each fill reservoir (1 through 8) of the TaqMan® Array Card is loaded with a sample-specific PCR reaction mix according to the card layout.

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.

   Do not create bubbles in the Master Mix.

2. Combine the cDNA and Master Mix in an appropriately-sized microcentrifuge tube according to the following table:

Component	Volume per fill reservoir [1]
cDNA sample + nuclease-free water	55 µL
Master Mix (2X)	55 µL
Total volume	**110 µL**

[1] Includes 10% overage.

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

Prepare a TaqMan® Array Card

IMPORTANT! Before preparing a TaqMan® Array Card, review *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Cards* (Pub. No. 4400263).

1. Load each fill reservoir of the card with 100 µL of prepared PCR reaction mix.
2. Centrifuge, then seal the filled card.

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 card.

Note: The instrument must be configured with a block appropriate for a card.

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.

3. Set up the thermal protocol for your instrument.

   See "Thermal protocols" on page 3 for the thermal protocols for other Master Mixes.

   Note: Your thermal protocols might differ from the following tables in this user guide.

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

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation[2]	92°C	10 minutes[3]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

[1] Optional, for optimal UNG activity.
[2] To activate AmpliTaq™ Fast DNA Polymerase.
[3] To completely dissolve the primers and probes on the card.

   Table 2 TaqMan® Fast Advanced Master Mix (7900HT Fast Real-Time PCR Instrument with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation[2]	92°C	10 minutes[3]	1
Denature	97°C	1 second	40
Anneal / Extend	60°C	20 seconds	

[1] Optional, for optimal UNG activity.
[2] To activate AmpliTaq™ Fast DNA Polymerase.
[3] To completely dissolve the primers and probes on the card.

4. Confirm that the reaction volume is set to 1 µL.
5. Load the card 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.

A cutoff of 32 is recommended. If pre-amplification is used, the cutoff can be set to 29 or 30 to reduce the number of false positives.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values (if using the baseline threshold method of analysis).
  - 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 the Relative Quantification application or ExpressionSuite™ Software.

C_t (Cq) values can be generated using the relative threshold algorithm (C_{rt}).

Use the relative threshold algorithm in your software. The relative threshold algorithm is available on the following instruments:

- QuantStudio™ Real-Time PCR Instruments
- ViiA™ 7 instrument

The Relative Quantification application is also available on the Thermo Fisher Cloud.

The C_t algorithm is recommended on the 7900HT Fast Real-Time PCR Instrument.

Algorithms for data analysis

Table 3 Algorithm recommendations for TaqMan® Array Cards

Algorithm	Recommendation
Relative threshold (C_{rt})	Recommended for the following instruments: • QuantStudio™ Real-Time PCR Instruments • ViiA™ 7 instrument Can correct a variable baseline, which might be due to dried-down assays on the card being reconstituted at different rates.
Threshold (C_t)	Optional if used for analysis of established protocols. Recommended for 7900HT Fast Real-Time PCR Instrument.

The relative threshold algorithm is available in the Relative Quantification application on the Thermo Fisher Cloud (**thermofisher.com/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.

Note: Your thermal protocols might differ from the following tables in this user guide.

Table 4 TaqMan® Gene Expression Master Mix and TaqMan® Universal Master Mix II, with UNG (ViiA™ 7 and compatible QuantStudio™ systems)

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

[1] Optional, for optimal UNG activity.

[2] To activate the enzyme and to completely dissolve the primers and the probes on the card.

Table 5 TaqMan® Gene Expression Master Mix and TaqMan® Universal Master Mix II, with UNG (7900HT Fast Real-Time PCR Instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
UNG incubation[1]	50.0°C	2 minutes	1
Enzyme activation	94.5°C	10 minutes[2]	1
Denature	97.0°C	30 seconds	40
Anneal / Extend	59.7°C	60 seconds	

[1] Optional, for optimal UNG activity.

[2] To activate the enzyme and to completely dissolve the primers and the probes on the card.

Table 6 TaqMan® Universal Master Mix II, no UNG (ViiA™ 7 and compatible QuantStudio™ systems)

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

[1] To activate the enzyme and to completely dissolve the primers and the probes on the card.

Table 7 TaqMan® Universal Master Mix II, no UNG (7900HT Fast Real-Time PCR Instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
Enzyme activation	94.5°C	10 minutes[1]	1
Denature	97.0°C	30 seconds	40
Anneal / Extend	59.7°C	60 seconds	

[1] To activate the enzyme and to completely dissolve the primers and the probes on the card.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

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Revision history: Pub. No. 4371129

Revision	Date	Description
C	20 November 2018	• Corrected thermal cycling conditions for the following Master Mixes: – TaqMan® Fast Advanced Master Mix – TaqMan® Gene Expression Master Mix – TaqMan® Universal Master Mix II, with UNG – TaqMan® Universal Master Mix II, no UNG • Removed TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG as a compatible Master Mix. • Updated algorithms for data analysis for 7900HT Fast Real-Time PCR Instrument.
B	10 July 2018	• Added thermal cycling protocols for all compatible Master Mixes. • Added procedural guidelines. • Added reference to user guide for detailed instructions to prepare the card. • Added new instruments, Master Mixes, and other applicable products. • Updated for general style, formatting, and branding.
A	February 2006	New document.