TaqMan™ Gene Expression Assays—TaqMan™ Array Cards

USER GUIDE

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ThermoFisher SCIENTIFIC

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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	Date	Description
G	8 March 2021	• Added the QuantStudio™ 7 Pro Real-Time PCR System and the QuantStudio™ Design and Analysis Software v2. • Updated centrifuges. • Updated the name of the cloud-based platform to Thermo Fisher™ Connect and updated the URLs.
F	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.
E	10 July 2018	• Updated recommended action if too much cDNA template is added. • Added information about recommended algorithms for data analysis.
D	5 March 2018	• Updated information to obtain files for the cards. • Added new instruments, Master Mixes, and other applicable products. • Added thermal cycling protocols for all compatible Master Mixes. • Updated guidelines for reverse transcription. • Removed content that is described in other resources; added references as appropriate. • Updated for general style, formatting, and branding.
C	July 2010	Baseline for this revision history.

Contents

1 Product information

Product description

The procedures in this document are for use with dried-down TaqMan™ Gene Expression Assays that are configured on TaqMan™ Array Cards.

TaqMan™ Gene Expression Assays are a comprehensive collection of predesigned, preformulated primer and probe sets to perform quantitative gene expression studies on various species.

For a current list of available species and assays, use the assay search tool at **thermofisher.com/taqmangeneexpression**.

- **TaqMan™ Gene Expression Assays**
  - Are a general collection of assays that target protein-coding transcripts from various species and for specific diseases, pathways, or biological processes.
  - Include TaqMan™ Non-coding RNA Assays that target long non-coding RNA (ncRNA) in human, mouse, and rat species. These assays are designed for ncRNAs that are >60 nt in length.
- **Endogenous control assays** (see page 32 for more information)

The following configurations are available:

Configuration	Description	Customizable
Fixed-content TaqMan™ Array Cards	Preplated and predefined TaqMan™ Gene Expression Assays that are manufactured and stocked in advance	No
Flexible-content TaqMan™ Array Cards	Cards configured with a suggested selection of TaqMan™ Gene Expression Assays, categorized by disease, pathway, or biological process	Preselected assays can be substituted with other predesigned assays that target TaqMan™ Gene Expression Assays more applicable to experiment needs
Custom-configured TaqMan™ Array Cards	Fully customizable cards	Allows the configuration of the TaqMan™ Array Cards with any predesigned assays

This document provides guidance for preparing cDNA templates (see page 12) and protocols for performing real-time PCR (qPCR) using a variety of compatible instruments and Master Mixes (see page 13 and page 36).

For more information about TaqMan™ Gene Expression Assays, see Appendix C, “Supplemental information”.

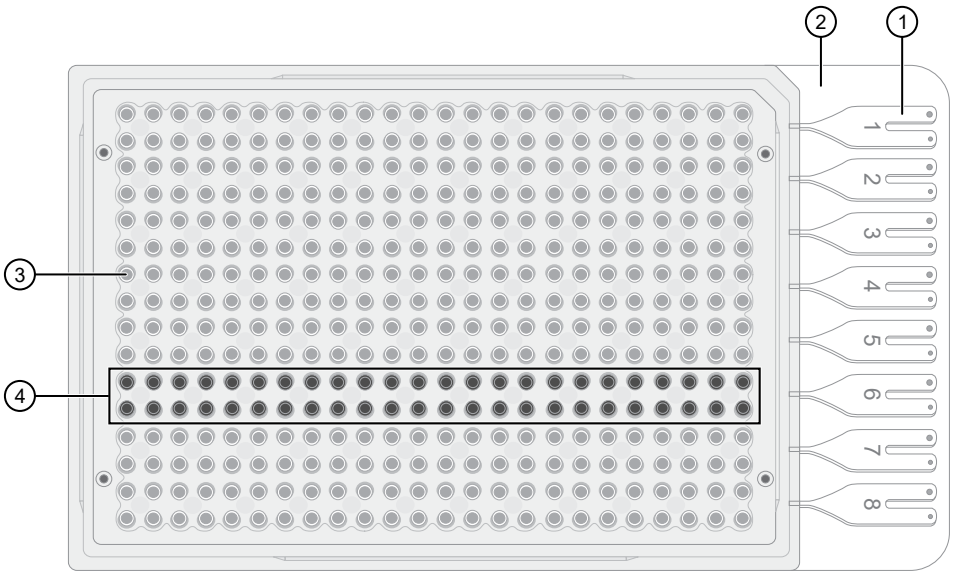
Over of a TaqMan™ Array Card

A TaqMan™ Array Card is a 384-well microfluidic card that is prepared with dried-down TaqMan™ Assays. With an array card, gene expression is measured using the comparative C_t ($\Delta\Delta C_t$) method of relative quantitation. For more information about relative quantitation, see the **Resources** section at **thermofisher.com/taqman**.

Advantages of a TaqMan™ Array Card include the following:

- Small-volume design that minimizes sample and reagent consumption.
- Streamlined reaction setup that saves time and reduces labor-intensive steps.
- Access to high-throughput, 384-well format without liquid-handling robotics.
- Two-fold discrimination detection at the 99.7% confidence level.
- Standardization across multiple samples in multiple laboratories.

Each card can run 1 to 8 samples against 12 to 384 TaqMan™ Assay targets (including controls).



(1) **Fill reservoir**—Each reservoir is loaded with a sample-specific PCR reaction mix; the associated reaction wells fill with that sample (8 total reservoirs)

(2) **Fill reservoir strip**—Support strip for fill reservoirs; removed before running the card

(3) **Reaction well**—Each well contains dried-down assay (384 total reaction wells)

(4) **Reaction well row**—A set of reaction wells that fill with the same sample-specific PCR reaction mix (8 total rows, each row associated with a single fill reservoir)

Contents and storage

TaqMan™ Array Cards contain dried-down TaqMan™ Gene Expression Assays according to the configured card layout.

Table 1 Available card layouts

Format	Cat. No.	Number of assays + controls	Number of samples	Storage[1]
TaqMan™ Array Card 12	4342247	11+ 1	8 quadruplicates	2–8°C[2]
TaqMan™ Array Card 16	4346798	15+ 1	8 triplicates	
TaqMan™ Array Card 24	4342249	23+ 1	8 duplicates	
TaqMan™ Array Card 32	4346799	31+ 1	4 triplicates	
TaqMan™ Array Card 48	4342253	47+ 1	8 no replicates	
TaqMan™ Array Card 64	4346800	63+ 1	2 triplicates	
TaqMan™ Array Card 96a	4342259	95+ 1	4 no replicates	
TaqMan™ Array Card 96b	4342261	95+ 1	2 duplicates	
TaqMan™ Array Card 192	4346802	191+ 1	1 duplicates	
TaqMan™ Array Card 384	4342265	380+ 4	1 no replicates	

[1] See packaging for expiration date.
[2] Shipped at ambient temperature. See thermofisher.com/ambientshippping.

Go to thermofisher.com/taqmanfiles to download the following files:

- **Card layout files (HTML and CSV)**—Show the position of each assay on the card. The HTML and CSV files contain identical information.
- **Setup files (SDS in TXT format)**—Contain the layout of the targets on the card. The files are imported into the software specific to your instrument to perform real-time PCR.
- **Assay Information File (AIF in TXT format)**—Describes the TaqMan™ Gene Expression Assays. See *Understanding Your Shipment* (Pub. No. MAN0017153) for detailed information about the Assay Information File.

Note: During the download, you may be asked to enter specific order numbers or product information.

The run properties and the thermal protocol are not defined in the setup files and must be set up in the instrument or software.

Order TaqMan™ Array Cards

- Order fixed- or flexible-content TaqMan™ Array Cards.
  a. Go to **thermofisher.com/taqmanarrays**.
  b. Search by disease, pathway, biological process, or gene symbol.
- Order custom-configured TaqMan™ Array Cards.
  a. Go to **thermofisher.com/customarraycards**.
  b. Select a format, then use the **Custom Array Configurator**.

Required materials and equipment not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Table 2 Recommended products for isolation of RNA

Item	Source
Kits for RNA isolation	**thermofisher.com/rnaisolation**

Table 3 Recommended products for preparation of cDNA

Item	Source
cDNA kit or cDNA Master Mix, one of the following:	
SuperScript™ IV VILO™ Master Mix	11756050
SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme	11766050
High-Capacity cDNA Reverse Transcription Kit	4368813

Table 4 PCR Master Mixes

Item	Source
(Recommended) TaqMan™ Fast Advanced Master Mix	4444556
TaqMan™ Gene Expression Master Mix	4369016
TaqMan™ Universal Master Mix II, with UNG	4440038
TaqMan™ Universal Master Mix II, no UNG	4440047

Table 5 Other materials and equipment required for the workflow

Item	Source
Real-time PCR instrument, one of the following:	
The instrument must be configured with the TaqMan™ Array Card block and heated cover.	
QuantStudio™ 7 Pro Real-Time PCR System	Contact your local sales office
QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	
Software	
QuantStudio™ Design and Analysis Software v2 For use with the QuantStudio™ 7 Pro Real-Time PCR System. Can be used to analyze data files from all of the other compatible instruments.	**thermofisher.com/qpcrsoftware**
(Optional) Relative Quantification application	Available at **thermofisher.com/connect**
(Optional) ExpressionSuite™ Software	Available at **thermofisher.com/expressionsuite**
Equipment	
Thermal cycler, one of the following (or equivalent): • Veriti™ Thermal Cycler • SimpliAmp™ Thermal Cycler • ProFlex™ PCR System	Contact your local sales office

Item	Source
Centrifuge with custom buckets and card holders, one of the following: • Sorvall™ centrifuge • Megafuge™ centrifuge • Multifuge™ centrifuge See the **Resources** section at **thermofisher.com/taqmanarrays** for a list of compatible centrifuges, rotors, and buckets.	Contact your local sales office
TaqMan™ Array Card Sealer (Referred to as Stylus Staker in some documents)	Contact your local sales office
Blank balance TaqMan™ Array Cards (Included with the instrument block upgrade / installation kit)	Contact your local sales office
Microcentrifuge	MLS
Vortex mixer	MLS
(Optional) Eppendorf™ MixMate™ (shaker)	Fisher-Scientific 21-379-00
Pipettes	MLS
Micropipettes	MLS
Tubes, plates, and other consumables	
Plastics consumables	**thermofisher.com/plastics**
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free water	MLS
RNase Inhibitor	N8080119
RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777019
TURBO DNA-*free*™ Kit	AM1907
DNase I, Amplification Grade	18068015

Workflow

Start with cDNA templates from RNA samples (page 12)

▼

Combine cDNA and Master Mix (page 14)

▼

Prepare a TaqMan™ Array Card (page 15)

(see page 25 for detailed procedures)

▼

Load the PCR reaction mix (page 27)

▼

Centrifuge the card (page 27)

▼

Seal the card (page 29)

▼

Set up and run the real-time PCR instrument (page 15)

▼

Analyze the results (page 16)

2 Guidelines for preparation of cDNA

Guidelines for isolation of high–quality RNA

- See Table 2 on page 8 for recommended RNA isolation kits.
- *(Optional)* Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparing cDNA templates

- See Table 3 on page 8 for recommended cDNA synthesis kits.
- 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

    Note: We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA (not applicable for double-stranded templates)

    IMPORTANT! Degradation of the RNA may reduce the yield of cDNA for some gene targets.
- 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.
- If using strip tubes to prepare cDNA templates, change to a new cap after each step or incubation.
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes to prepare cDNA templates.

3 Perform real-time PCR

Procedural guidelines for performing real-time PCR

- Follow best-practices when preparing or performing PCR (see page 38).
- 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.
- Before preparing a TaqMan™ Array Card, review Appendix B, “Detailed procedures for preparation of a TaqMan™ Array Card”.
- 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.
- Configure run documents according to the instructions provided in the real-time PCR instrument resource documents.
- 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.
- Run the card within 72 hours of sealing the card if the following Master Mixes are used:
  - TaqMan™ Fast Advanced Master Mix
  - TaqMan™ Gene Expression Master Mix
- Run the card within 24 hours of sealing the card if the following Master Mixes are used:
  - TaqMan™ Universal Master Mix II, with UNG
  - TaqMan™ Universal Master Mix II, no UNG
- If the card is not run immediately, protect it from light and store at 2-8°C.

Guidelines for quantity of cDNA

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).

The recommended quantity of cDNA depends on the expression level of the target genes and the number of target copies per well.

Gene expression level	Quantity of cDNA per fill reservoir
Low expression	1,000 ng (10 ng/µL)[1]
Moderate expression	100–200 ng (1–2 ng/µL)
Moderate to high expression	30–50 ng (0.3–0.5 ng/µL)

[1] The cDNA concentration will be high, so ensure that the cDNA is high-quality and without inhibitors.

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 Appendix B, "Detailed procedures for preparation of a TaqMan™ Array Card".

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.
   See page 35 for instructions on how to import setup files.

   Note: The setup files are instrument–specific. See page 35 for more information.

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 36 for the thermal protocols for other Master Mixes.

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

Table 6 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 7 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 any of the following software:

Software	Resource
Relative Quantification application	**thermofisher.com/connect**
ExpressionSuite™ Software[1]	**thermofisher.com/expressionsuite**

[1] Can automatically define the baseline.

For more information about real-time PCR, see page 43 or go to **thermofisher.com/qpcreducation**.

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 Thermo Fisher™ Connect.

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

See *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239) for information about C_t and C_{rt}.

Algorithms for data analysis

Table 8 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 Thermo Fisher™ Connect (**thermofisher.com/connect**).

A

Troubleshooting

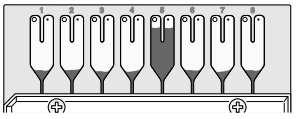
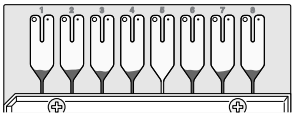
Troubleshooting: After removing the card from packaging

Observation	Possible cause	Recommended action
Water condenses on the reaction wells (optical side of the card)	The card was not at room temperature before being removed from the packaging.	Remove condensation on the reaction wells by lightly blowing room temperature pressurized nitrogen or an air blower on the wells. **IMPORTANT!** Ensure that all water condensation is removed. The optical side of the card must be free of water condensation.

Troubleshooting: After loading PCR reaction mix into the card

Observation	Possible cause	Recommended action
Fill reservoir is not full of PCR reaction mix	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.
PCR reaction mix leaks from the vent port into the fill reservoir	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.
Fill reservoirs have bubbles in the PCR reaction mix	When loading the card with PCR reaction mix, air was introduced into the fill reservoir.	Inspect the affected rows after centrifuging and sealing the card. Note wells that contain bubbles, then consider omitting these wells from analysis.

Troubleshooting: After centrifuging the card

Observation	Possible cause	Recommended action
PCR reaction mix remains in a fill reservoir 	Though rare, the fill port is blocked.	Inspect the card for blocked fill port or a pinched channel. If the fill reservoir is defective, contact Support.
	Filling is incomplete or not consistent.	Centrifuge the card again for 1 minute.
		If the filling is still incomplete after the additional centrifuge cycle, continue with running the card. However, consider omitting the wells associated with that fill reservoir.
Fill reservoir completely empty 	Some wells were filled improperly.	Continue with running the card. However, consider omitting the wells associated with that fill reservoir.

Troubleshooting: After running the card and reviewing run results

Observation	Possible cause	Recommended action
A gradient signal across the card	The card was in a diagonal position during centrifugation because not all of the positions in the card holder were filled.	Repeat the assay with a new card. Ensure that all the positions in the centrifuge card holder are filled.
Noise in the amplification plots for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  ① Array card feet
No amplification for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  ① Array card feet

Observation	Possible cause	Recommended action
No amplification in some wells	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer. **IMPORTANT!** Do not move the carriage back across the card. See "Seal the card" on page 29.
No amplification within or across one or more rows	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer.
	Empty wells due to misalignment of the TaqMan™ Array Card Sealer.	If the TaqMan™ Array Card Sealer is misaligned, contact Support.
	PCR reaction mix improperly prepared.	Ensure that all reaction components were added to the PCR reaction mix.
Replicates have poor precision **Details:** Poor precision is when the standard deviation value is > 0.5 for assays with a C_t value <30.	Bubbles in wells.	Use proper pipetting techniques to avoid introducing air into the fill reservoirs. Consider omitting the leaking wells from analysis.
	Wells leaking due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer. Consider omitting the leaking wells from analysis. **IMPORTANT!** Do not move the carriage back across the card. See "Seal the card" on page 29.
	Not all of the positions in the card holder were filled before centrifuging.	Ensure that all of the empty positions of the card holder are filled with blank balance cards before centrifuging.
	The cards were centrifuged using a non-verified centrifuge.	Use a verified centrifuge (see "Required materials and equipment not supplied" on page 8). See the **Resources** section at thermofisher.com/taqmanarrays for a list of compatible centrifuges.
	The dried-down assays on the card were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Analyze results with the relative threshold algorithm (C_{rt}) instead of the baseline threshold algorithm (C_t).
The baseline is variable	The dried-down assays on the card were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Use the relative threshold algorithm (C_{rt}). C_{rt} can correct for a variable baseline.
		Use the Relative Quantification application, available on Thermo Fisher™ Connect. The Relative Quantification application uses C_{rt} if the software specific to your instrument does not have the relative threshold algorithm.

Observation	Possible cause	Recommended action
The Multicomponent Plot shows low ROX™ dye (passive reference dye)	Little or no Master Mix is present due to inaccurate pipetting.	Follow accurate pipetting practices.
	The card was sealed before it was centrifuged.	Ensure that the card is centrifuged before sealing. See Appendix B, "Detailed procedures for preparation of a TaqMan™ Array Card".
Amplification curve shows no amplification of the sample (C_t=40) in the target assay	The gene is not expressed in the tested sample.	• Ensure that the gene is expressed in the sample type or tissue type. • Confirm the results. – Rerun the sample using the same assay in a single-tube format. – Rerun the experiment using more sample. Avoid preparing PCR reaction mixes with more than 20% reverse transcription reaction. – Run the experiment using an alternative assay, if available, that detects a different transcript or more than one transcript from the same gene. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	The sample does not have enough copies of the target RNA.	Confirm the results. • Rerun the sample using the same assay. • Rerun the assay using more sample. Avoid PCR reaction mix with more than 20% from the reverse transcription reaction. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	One or more of the reaction components was not added. On a TaqMan™ Array Card, the full reaction well row will show no amplification.	Check your pipetting equipment and/or technique.
	Incorrect dyes were selected for each target. On a TaqMan™ Array Card, the full reaction well row will show no amplification.	Check the dyes selected for the run, then reanalyze the data.
Decrease in ROX™ dye fluorescence (passive reference dye)	There was precipitation in the buffers.	Mix the Master Mix thoroughly to produce a homogenous solution.
	The reagents are degraded.	Ensure that the kits and reagents have been stored according to the instructions on the packaging and that they have not expired.

Observation	Possible cause	Recommended action
The multicomponent signal for ROX™ dye is not flat	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.
The Rn in the Rn vs Cycle plot is very high	The ROX™ dye was not set as the passive reference.	Set ROX™ dye as the passive reference, then reanalyze the data.
The no template control (NTC) shows amplification	The reagents are contaminated with gDNA, amplicon, or plasmid clones.	• Rerun the assay using new reagents. • Ensure that your workspace and equipment are cleaned properly. • Use a Master Mix that contains UNG. • Run no-RT controls to rule out genomic DNA contamination. • Treat the sample with DNase.
The endogenous control C_t values vary or they do not normalize the sample well	The endogenous control is not consistently expressed across the samples.	Ensure that the endogenous control is consistently expressed in your sample type. See "Endogenous controls" on page 32.
	The sample concentrations vary.	Quantitate and normalize the PCR samples.
	Pipetting was inaccurate.	• Check the calibration of the pipettes. • Pipet at least 5 µL of sample to prepare the reaction mix.
There is a high standard deviation in the replicates, inconsistent data, or a variable C_t	The reagents were not mixed properly.	• Increase the length of time that you mix the reagents. • Verify your mixing process by running a replicate assay.
	Pipetting was inaccurate.	• Check the calibration of the pipettes. • Pipette at least 5 µL of sample to prepare the reaction mix.
	The threshold was not set correctly.	Set the threshold above the noise level and where the replicates are tightest. See your real-time PCR system user documentation.
	There was a low concentration of the target of interest.	Rerun the assay using more cDNA template.
The C_t value is lower than expected	Genomic DNA (gDNA) contamination occurred.	• Run a no-RT control to confirm that there was gDNA contamination. • Use DNase to ensure minimal gDNA contamination of the RNA.
	Too much cDNA template was added.	• Reduce the amount of cDNA template. • Quantitate the RNA before the RT reaction, then adjust the concentration of cDNA from the RT reaction that is added to the real–time PCR reaction.
	The cDNA template or the amplicon is contaminated.	Follow established PCR good laboratory practices.

Observation	Possible cause	Recommended action
Amplification occurs in the no-RT controls	Genomic DNA (gDNA) contamination occurred.	• Improve sample extraction methods to eliminate gDNA. See Chapter 2, "Guidelines for preparation of cDNA". • Use DNase to ensure minimal gDNA contamination of the RNA.
	The cDNA template or amplicon is contaminated.	Follow established PCR good laboratory practices.
There was a shifting Rn value during the early cycles of the PCR (cycles 0 to 5)	Fluorescence did not stabilize to the buffer conditions of the reaction mix. **Note:** This condition does not affect PCR or the final results.	• Reset the lower value of the baseline range. • Use an automatic baseline. • Use the relative threshold algorithm (C_{rt}). See *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239).
There was a small ΔRn	The PCR efficiency was poor.	Ensure that the reagents were used at the correct concentration.
	The quantity of the cDNA is low (a low copy number of the target).	Increase the quantity of the cDNA.

B

Detailed procedures for preparation of a TaqMan™ Array Card

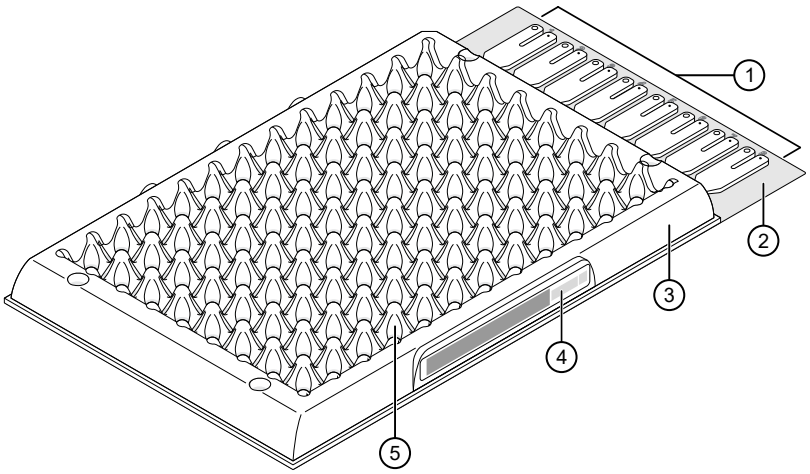
Guidelines for preparation of a card

- 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.
- Before removing the card from its packaging:
  - Prepare each sample-specific PCR reaction mix.
  - Allow the card to reach room temperature.
- 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.
- Do not allow the micropipette tip to contact the coated foil beneath the fill port.

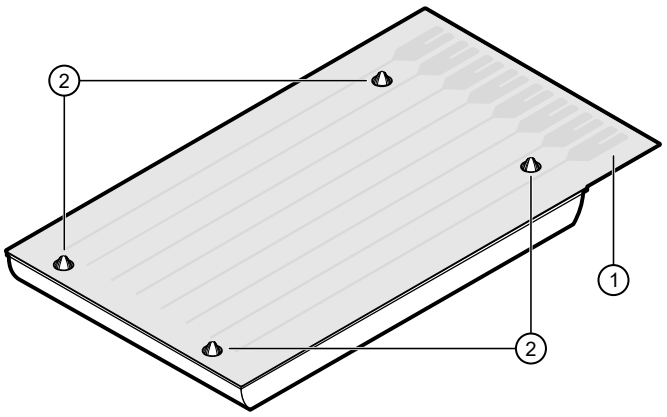
- Load the card with PCR reaction mix *before* centrifuging the card.
  During centrifugation, the PCR reaction mix resuspends the dried-down assays in each well of the card. Adding sample after centrifuging disrupts the assay layout of the card.
- Run the card within the time allowed by the Master Mix. See “Procedural guidelines for performing real-time PCR” on page 13.
- If the card is not run immediately, protect it from light and store at 2-8°C.

TaqMan™ Array Card diagram

A TaqMan™ Array Card includes 8 fill reservoirs and 384 reaction wells.

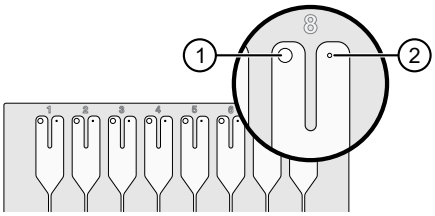


1. Fill reservoirs (8 total)
2. Fill reservoir strip
3. Array card carrier
4. Array card barcode
5. Reaction well (384 total)



1. Foil
2. Array card feet

The fill reservoir includes a fill port and a vent port. Use the fill port to load PCR reaction mix into the card.



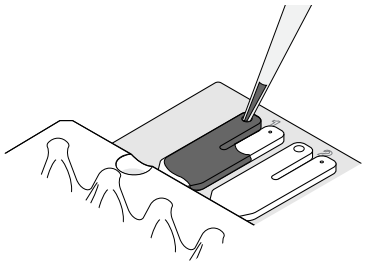
① Fill port
② Vent port

Load the PCR reaction mix

Before removing the card from its packaging:

- Prepare each sample-specific PCR reaction mix.
- Allow the card to reach room temperature.

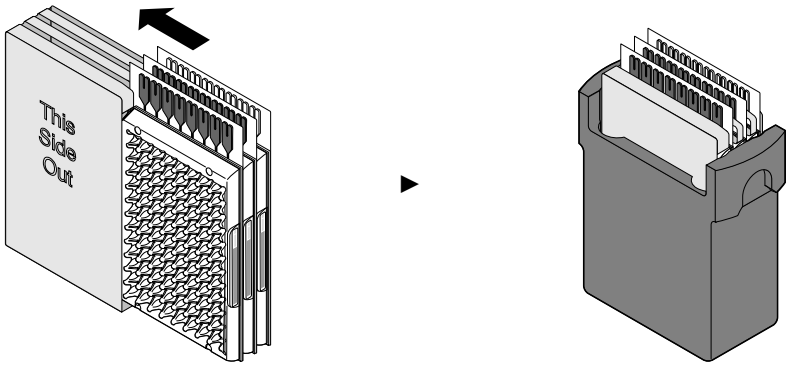
1. Carefully remove the card from its packaging.
2. Place the card on the benchtop with its foil-side down.
3. Load 100 µL of the sample-specific PCR reaction mix into a micropipette.
4. Hold the micropipette in an angled position, then place the tip into a fill port of the card.
5. Slowly dispense the entire volume of reaction mix so that it sweeps in and around the fill reservoir toward the vent port.



Centrifuge the card

1. Load the cards into the centrifuge buckets.
   a. Place the bucket on the benchtop with its label facing the front of the bench.
   b. Insert the cards into the card holder, ensuring that:
      - The fill reservoirs extend upwards out of the card holder.
      - The reaction wells face the label-side of the card holder.
   c. Insert blank balance cards into any empty positions of the card holder. All three positions in the card holder must be filled.

d. Place the loaded card holder into the bucket so that the card holder label faces the front of the bucket.



2. Configure the centrifuge using its front-panel controls.

   a. Set the bucket type to **15679**.

   b. Set the following parameters according to the control panel type.

Parameter	EASYSet (touchpad-operated)	QUIKSet (knob-operated)
Increasing ramp rate	9	3
Decreasing ramp rate	9	—
Rotational speed	1,200 rpm (331 × *g*)	1,200 rpm
Centrifugation time [1]	1 minute	1 minute

[1] You will centrifuge the cards twice, each time for 1 minute (see step 4).

IMPORTANT! A speed that is set too high can deform the card.

3. Load the buckets into the centrifuge.

   a. Press ⏏ on the centrifuge control panel to open the centrifuge cover.

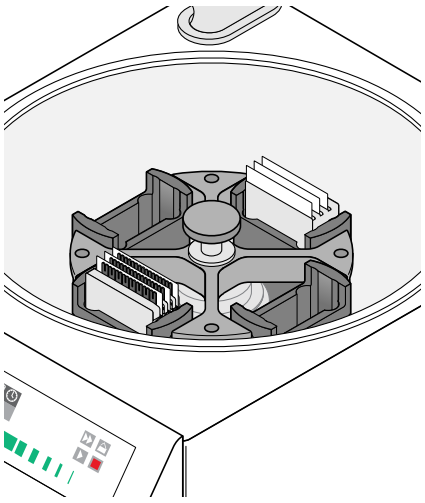
   b. Place each loaded bucket onto an open rotor arm of the centrifuge.

   Ensure that each bucket can swing easily within its slotted position on the rotor arm.

   c. If there are empty rotor arms, prepare buckets with blank balance cards as described in step 1. Place the balance buckets onto the rotor arms.

   The rotor must be evenly loaded and opposing buckets must hold the same weight.

   d. Close the centrifuge cover.



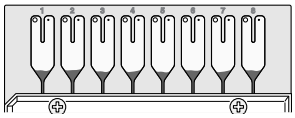
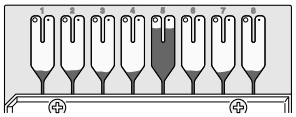
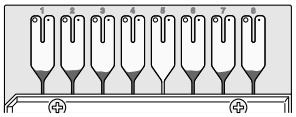
Centrifuge is properly loaded and balanced.

4. Run the centrifuge.
   a. Press ▶.
      The centrifuge will start, then automatically stop after 1 minute.
   b. Repeat substep 4a so that the cards are centrifuged for a total of two, consecutive, 1-minute centrifugations.

      IMPORTANT! Do not centrifuge the cards continuously for 2 minutes. The ramping up in speed during the *two, consecutive 1-minute* centrifugations is important for proper filling.

5. Remove the cards from the centrifuge.
   a. Press ⏏.
   b. Remove the buckets from the centrifuge, then remove the card holders from the buckets.
   c. Remove each card from the card holder by lifting it gently by the card carrier sides.

6. Examine the cards for proper filling.
   When properly filled, the remaining volumes of PCR reaction mix are consistent reservoir to reservoir.

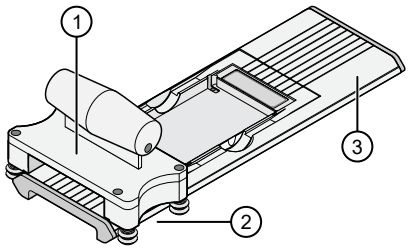
Consistent filling	Inconsistent filling	
		

Seal the card

The TaqMan™ Array Card Sealer isolates the wells of an array card after it is loaded with PCR reaction mix and centrifuged. The sealer uses a precision stylus assembly (under the carriage) to seal the main fluid distribution channels of the array card.

Note: In some documents, the TaqMan™ Array Card Sealer is referred to as a Stylus Staker.

1. Position the TaqMan™ Array Card Sealer and its carriage before inserting a card.
   a. Place the sealer on a benchtop that is approximately waist-high so that the carriage can be easily maneuvered.
   b. Position the sealer with the carriage starting position toward the front of the bench.
      Ensure that the engraved arrows on the sealer point away from you.



① Carriage
② Carriage starting position
③ Carriage ending position

c. Ensure that the carriage for the sealer is at the starting position.

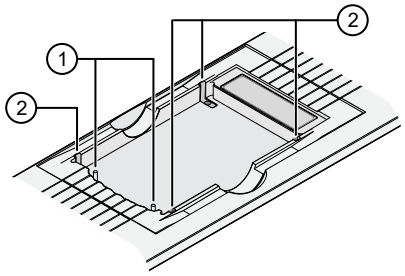
IMPORTANT! Do not insert a filled card into the sealer if the carriage is *not* in its starting position. The card will be irreparably damaged if the carriage is moved backwards across the card towards the starting position.

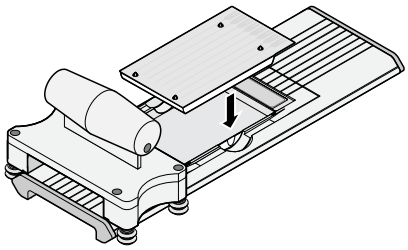
2. Insert a filled, centrifuged card into the sealer.

a. Hold the card with its foil-side up.

b. Orient the card over the sealer with the fill reservoirs of the card toward the ending position.

c. Align the rear pin grooves of the card to the alignment pins of the sealer.



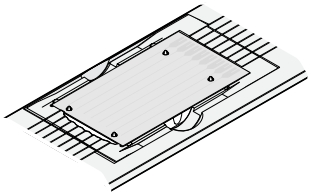


① Alignment pins
② Spring clips

d. Gently place the card on top of the sealer.

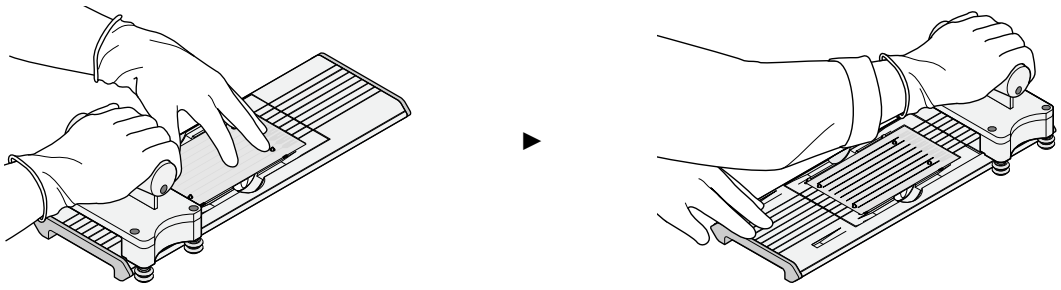
e. Gently push the card until it is seated securely in the sealer.

When properly seated, the foil surface of the card is level with the base of the sealer and the spring clips hold the card securely in place.



3. Slowly and steadily push the carriage across the sealer in the direction of the engraved arrows.

Push the carriage across the entire length of the card until the carriage meets the mechanical stops at the ending position.



IMPORTANT!

- Do not use excessive force or speed when pushing the carriage across the card.
- Do not move the carriage back across the card. Leave the carriage at the ending position while removing the card from the sealer.

4. Remove the sealed card from the sealer by grasping the sides of the card and lifting it off.

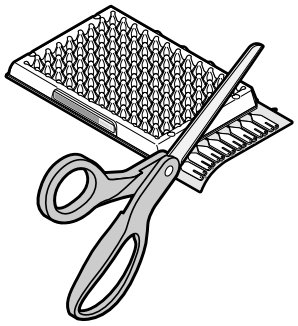
   Use the thumb slot in the middle of the sealer to access the card.

5. Examine the card for proper sealing.

   Note: When properly sealed, the indentations from the sealer carriage align with the main channels of the card.

   If the indentations do not align or if the foil is damaged, do not use the card.

6. Use scissors to trim the fill reservoir strip from the card. Use the edge of the card carrier as a guide.



IMPORTANT! Completely remove the fill reservoir strip. Any remaining plastic that extends beyond the card edge can prevent the card from seating properly on the sample block and can affect amplification.

Correct trim	Incorrect trim
	

The card is now ready to run on the instrument.

(Optional) Store the sealed card, protected from light, at 2–8°C. The length of time the card can be stored depends on the Master Mix. See “Procedural guidelines for performing real-time PCR” on page 13.

C Supplemental information

Endogenous controls

An endogenous control shows gene expression that is relatively constant and moderately abundant across tissues and cell types and treatment protocols. Normalization to endogenous control genes is currently the most accurate method to correct for potential biases that are caused by:

- Sample collection
- Variation in the amount of starting material
- Reverse transcription (RT) efficiency
- Nucleic acid (RNA/DNA) preparation and quality

No single control can act as a universal endogenous control for all experimental conditions, so we recommend verifying the chosen endogenous control or set of controls for the sample tissue, cell, or treatment. See *Using TaqMan™ Endogenous Control Assays to select an endogenous control for experimental studies* (Pub. No. COL33019 0619), available from **thermofisher.com**.

To select and order endogenous control assays, go to **thermofisher.com/taqmancontrols**.

Candidate endogenous controls

Gene symbol	Gene name	Human assay ID	Mouse assay ID	Rat assay ID
18S	Eukaryotic 18S rRNA	Hs99999901_s1	Hs99999901_s1	Hs99999901_s1
ACTB	Actin, Beta, cytoplasmic	Hs99999903_m1	Mm00607939_s1	Rn00667896_m1
B2M	Beta-2-microglobulin	Hs99999907_m1	Mm00437762_m1	Rn00560865_m1
GAPDH	Glyceradehyde-3-phosphate dehydrogenase	Hs99999905_m1	Mm99999915_g1	Rn99999916_s1
GUSB	Beta glucuronidase	Hs99999908_m1	Mm00446953_m1	Rn00566655_m1

(continued)

Gene symbol	Gene name	Human assay ID	Mouse assay ID	Rat assay ID
HMBS	Hydromethylbilane synthase	Hs00609297_m1	Mm00660262_g1	Rn00565886_m1
HPRT1	Hypoxanthine guanine phosphoribosyl transferase 1	Hs99999909_m1	Mm00446968_m1	Rn01527840_m1
IP08	Importin 8	Hs00183533_m1	Mm01255158_m1	Not Available
PGK1	Phosphoglycerate kinase 1	Hs99999906_m1	Mm00435617_m1	Rn00821429_g1
POLR2A	Polymerase (RNA) II (DNA directed) polypeptide A, 220 kDa	Hs00172187_m1	Mm00839493_m1	Rn01752026_m1
PPIA	Peptidylprolyl isomerase A	Hs99999904_m1	Mm02342430_g1	Rn00690933_m1
RPLP0	Ribosomal protein, large, P0	Hs99999902_m1	Mm00782638_s1	Rn01479927_g1
TBP	TATA box binding protein	Hs99999910_m1	Mm00446973_m1	Rn01455648_m1
TFRC	Transferrin receptor	Hs99999911_m1	Mm00441941_m1	Rn01474695_m1
UBC	Ubiquitin C	Hs00824723_m1	Mm01201237_m1	Rn01789812_g1
YWHAZ	Tyrosine 3-monooxygenase, or tryptophan 5-monooxygenase activation protein, zeta polypeptide	Hs00237047_m1	Mm01158417_g1	Rn00755072_m1

TaqMan™ Gene Expression Assays chemistry overview

TaqMan™ MGB probes

TaqMan™ MGB probes contain:

- A reporter dye (for example, FAM™ dye) at the 5′ end of the probe.
- A non-fluorescent quencher (NFQ) dye at the 3′ end of the probe.

  The NFQ dye does not fluoresce, which allows the real-time PCR system to measure the reporter dye contributions more accurately.
- A minor groove binder (MGB) at the 3′ end of the probe that:
  - Increases the melting temperature (T_m) without increasing the probe length.
  - Allows for the design of shorter probes.

About the 5' nuclease assay

Note: The following figures are general representations of real-time PCR with TaqMan™ MGB probes and TaqMan™ Gene Expression Assays. The sequence regions are not necessarily drawn to scale.

The 5' nuclease assay process takes place during PCR amplification. It occurs in every cycle and does not interfere with the exponential accumulation of cDNA synthesis product.

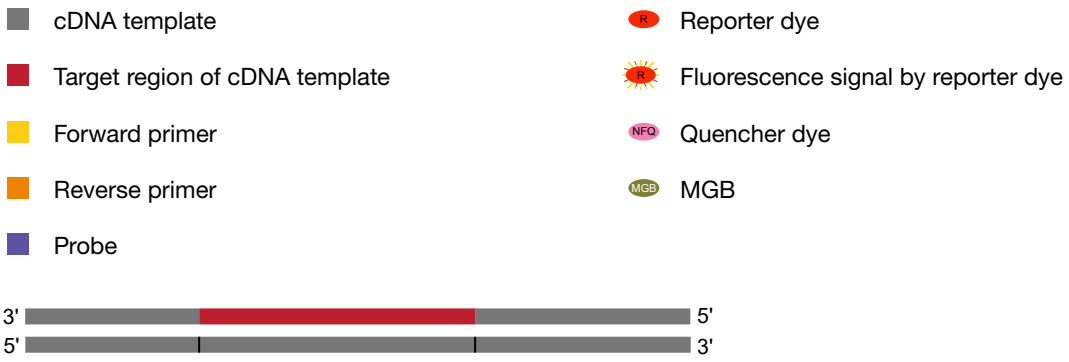


Figure 1 cDNA synthesis product

During the PCR, the forward and reverse primers anneal to complementary sequences along the denatured cDNA template strands (see Figure 2).

The TaqMan™ MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites (see Figure 2). When the probe is intact, the proximity of the reporter dye and quencher dye suppresses the reporter fluorescence, primarily by Förster-type energy transfer.

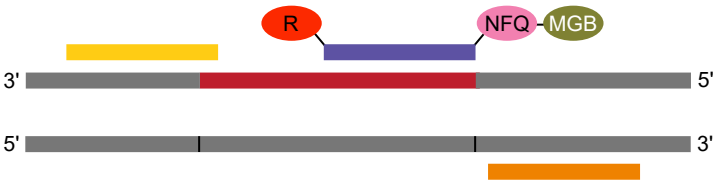


Figure 2 Annealing of probes and primers to cDNA strands

During polymerization, the DNA polymerase only cleaves probes that hybridize to the target sequence. Cleavage separates the reporter dye from the probe. The separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter dye (see Figure 3).

This increase in fluorescence occurs only if the probe is complementary to the target sequence and if the target sequence is amplified during PCR. Because of these conditions, nonspecific amplification is not detected.

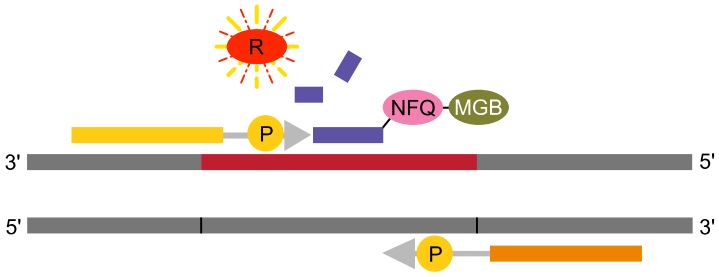


Figure 3 Initial polymerization and cleavage of reporter dye

Polymerization of the strand continues (see Figure 4), but because the 3' end of the probe is blocked, no extension of the probe occurs during PCR.

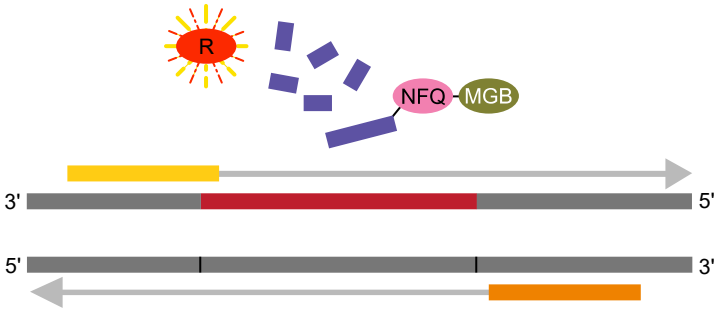


Figure 4 Completion of polymerization

Instrument software and setup files

Instrument software	Setup file
QuantStudio™ 7 Flex System	`SDS_ordernumber.txt`
QuantStudio™ 12K Flex Real–Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	

How to import setup files

Instrument	Navigation
QuantStudio™ 7 Pro Real-Time PCR System	1. In QuantStudio™ Design and Analysis Software v2, navigate to the **Plate Setup** tab and the plate layout pane. 2. Click ··· **(Actions)** ▸ **Import Plate Setup**. 3. Navigate to, then select the file. 4. Click **Open**.

(continued)

Instrument	Navigation
QuantStudio™ 7 Flex System	In the menu bar during setup, select **File ▸ Import Plate Setup**.
QuantStudio™ 12K Flex Real–Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	In the menu bar, select **File ▸ New Plate Wizard**, then follow the prompts.

Thermal protocols

The thermal protocol settings depend on:

- The real-time PCR instrument.
- Compatibility of the Master Mix with standard or fast cycling mode.
- Whether the Master Mix contains UNG.

The thermal protocols in "Set up and run the real-time PCR instrument" on page 15 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 9 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 10 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 11 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 12 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.

Best practices for PCR and RT-PCR experiments

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Use UNG to prevent false-positive amplification

Carryover amplicons can result in false-positive amplification during PCR. Use a Master Mix that contains uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)) to degrade many contaminating carryover amplicons.

UNG enzymatic activity occurs during an initial incubation at 50°C. UNG is partially inactivated during the 95°C incubation step for template denaturation and polymerase activation. Because UNG is not completely deactivated during the 95°C incubation, it is important to keep the annealing temperatures greater than 55°C and to refrigerate PCR products at 2°C to 8°C in order to prevent amplicon degradation.

To ensure the desired UNG activity:

- Use PCR components and thermal cycling conditions as specified.

  UNG-containing Master Mixes incorporate the optimal concentration of UNG to prevent cross-contamination while not affecting real-time PCR performance.
- Do not attempt to use UNG-containing Master Mixes in subsequent amplification of dU-containing PCR products, such as in nested-PCR protocols. The UNG will degrade the dU-containing PCR products, preventing further amplification.

Although treatment with UNG can degrade or eliminate large numbers of carryover PCR products, use good laboratory practices to minimize cross-contamination from non-dU-containing PCR products or other samples.

Detect fluorescent contaminants

Fluorescent contaminants can generate false positive results. To help detect these contaminants, we recommend including a no-amplification control reaction that contains sample, but no master mix.

After PCR, if the absolute fluorescence of the no-amplification control is greater than the fluorescence of the no template control (NTC), fluorescent contaminants may be present in the sample or in the heat block of the real-time PCR instrument.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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Documentation and support

Related documentation

Document	Pub. No.
TaqMan™ Gene Expression Assays Quick Reference—TaqMan™ Array Cards	4371129
Introduction to Gene Expression Getting Started Guide	4454239
Understanding Your Shipment (for detailed information about the Assay Information File)	MAN0017153
Applied Biosystems™ Relative Quantitation Analysis Module User Guide	MAN0014820
QuantStudio™ Design and Analysis Software v2	
QuantStudio™ Design and Analysis Software v2 User Guide	MAN0018200
QuantStudio™ 7 Pro Real-Time PCR System	
QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System User Guide	MAN0018045
QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ Real-Time PCR Software Getting Started Guide	4489822
QuantStudio™ 12K Flex Real–Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050
ViiA™ 7 Real-Time PCR System	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security	4442661
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide	4441434
7900HT Real-Time PCR System	
Applied Biosystems™ 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide	4365542
Applied Biosystems™ 7900HT Fast Real-Time PCR System User Bulletin	4352533

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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