TaqMan® Gene Expression Assays—TaqMan® Array Plates

USER GUIDE

384–well Specialty TaqMan® Array Plates

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

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A	24 May 2019	New document.

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1 Product information

Product description

The procedures in this document are for use with TaqMan® Gene Expression Assays configured and preplated on 384–well Specialty TaqMan® Array Plates.

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

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

- **TaqMan® Gene Expression Assays**
  - A general collection of assays that target protein-coding transcripts from a variety of species and for specific diseases, pathways, or biological processes.
  - 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 20 for more information)

This document provides guidance for preparing cDNA templates (see Chapter 2, "Guidelines for preparation of cDNA") and protocols for performing real-time PCR using a variety of compatible instruments and Master Mixes (see page 10 and "Thermal protocols" on page 23).

For detailed information about TaqMan® Gene Expression Assays, see Appendix B, "Supplemental information".

Contents and storage

Specialty TaqMan® Array Plates contain dried-down Custom TaqMan® Gene Expression Assays. Store plates at 15–30°C upon receipt.

Order Specialty TaqMan® Array Plates

Use the online configurator tool at **specialty-taqman-arrays.com**.

Use the following steps to order if your plates require duplexed assays, or assays ordered through our specialty oligos service.

1. Go to **thermofisher.com/customplating**.
2. Download, then complete the Custom Plating Service Form.
3. Email the completed form to **Specialty_Plates@thermofisher.com**.

Required materials and equipment not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 1 Recommended products for isolation of RNA

Item	Source
Kits for RNA isolation	thermofisher.com/rnaisolation

Table 2 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 3 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
TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG	4352042

Table 4 Other materials and equipment required for the workflow

Item	Source
Real-time PCR instrument, one of the following:	
QuantStudio™ 5 Real-Time PCR System	Contact your local sales office
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
Software	
(Optional) Relative Quantification application	Available on the Connect platform
(Optional) ExpressionSuite™ Software	Available at **thermofisher.com/expressionsuite**
Equipment	
Centrifuge, with adapter for 384-well plates	MLS
Microcentrifuge	MLS
Vortex mixer	MLS
(Optional) Eppendorf™ MixMate™ (shaker)	Fisher Scientific™ 21-379-00
Pipettes	MLS
Tubes, plates, and other consumables	
Tubes, plates, and film	**thermofisher.com/plastics**
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free Water	AM9930
RNase Inhibitor	N8080119
RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777019
TURBO DNA-*free*™ Kit	AM1907
(Optional) TaqMan® PreAmp Master Mix	4391128
(Optional) TaqMan® PreAmp Master Mix Kit	4384267

Workflow

Start with cDNA templates from RNA samples (page 9)

▼

Combine cDNA and Master Mix (page 11)

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Prepare the TaqMan® Array Plate (page 11)

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Set up and run the real-time PCR instrument (page 12)

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Analyze the results (page 13)

2 Guidelines for preparation of cDNA

Guidelines for isolation of high–quality RNA

- See Table 1 for recommended RNA isolation kits.
- *(Optional)* Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparing cDNA templates

- See Table 2 on page 6 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 "Best practices for PCR and RT-PCR experiments" on page 25).
- 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.
- Keep the plate 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 plate.

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, according to the following table.

Component	Volume per reaction [1]	
	Reaction volume 10 µL	Reaction volume 5 µL
cDNA sample + nuclease-free water[2]	5 µL	2.5 µL
Master Mix (2X)[3]	5 µL	2.5 µL
Total PCR Reaction Mix volume	**10 µL**	**5 µL**

[1] Add 10% overage for pipetting loss.
[2] Ensure that the final cDNA concentration per well is 10–100 ng per 10 µL or 5 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.
   - 10 µL reaction volume: 10 µL per well
   - 5 µL reaction volume: 5 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

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. Set up samples and targets on 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.

   See "Thermal protocols" on page 23 for the thermal protocols for other Master Mixes.

   Table 5 TaqMan® Fast Advanced Master Mix

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" on page 22.

4. Set the reaction volume.
   - 10 µL
   - 5 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 application	**thermofisher.com/connect**
ExpressionSuite™ Software[1]	**thermofisher.com/expressionsuite**

[1] Can automatically define the baseline.

For more information about real-time PCR, see "Procedural guidelines for performing real-time PCR" on page 10) or go to **thermofisher.com/qpcreducation**.

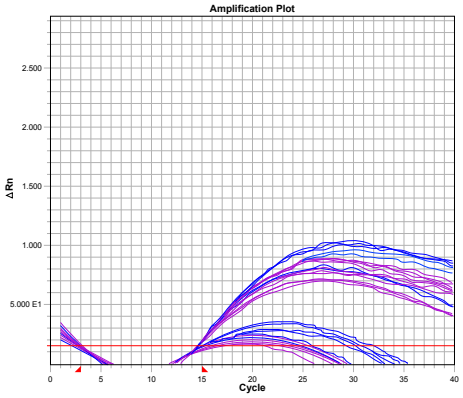
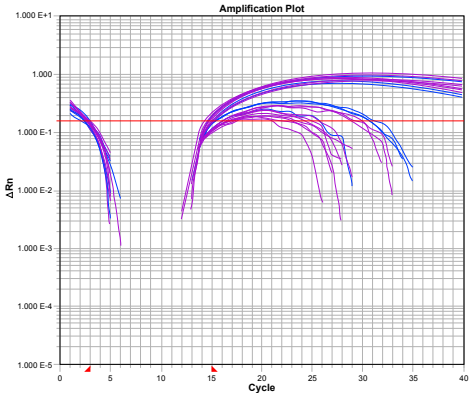
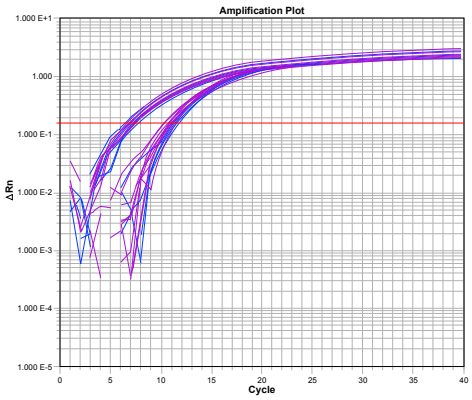
Data can be analyzed using the relative threshold algorithm (C_{rt}).

See "Algorithms for data analysis" on page 23.

Use the relative threshold algorithm in your software. If your software does not have the relative threshold algorithm, you can use the Relative Quantification application that is available on Connect (**thermofisher.com/connect**).

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

Troubleshooting

Observation	Possible cause	Recommended action
Amplification curve shows abnormal plot and/or low ΔRn values Linear view:  Log view:  	The baseline was set improperly (some samples have C_t values lower than the baseline stop value).	See your real-time PCR system user guide for procedures on setting the baseline. Switch from manual to automatic baselining, or move the baseline stop value to a lower C_t (2 cycles before the amplification curve crosses the threshold). Corrected log view: 
	An amplification signal is detected in the early cycles (no baseline can be set because the signal is detected too early).	Dilute the sample to increase the C_t value.
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.

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (C_t=40) across all assays or in an unusually large number of assays	One or more of the reaction components was not added.	Ensure that the cDNA and the Master Mix were added to the reaction plate. The passive reference fails if the Master Mix is missing.
	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.
	The annealing temperature was too high for the primers and/or probe.	Ensure that the correct annealing and extension temperatures are set.
		Ensure that the real-time PCR instrument is calibrated and maintained regularly.
	Inappropriate reaction conditions were used.	Troubleshoot the real-time PCR optimization.
		Ensure that the properties and the thermal protocol are correct.
	The template is degraded.	• Determine the quality of the template. • Rerun the assay with fresh template. • Use RNase-free reagents. • Use an RNase inhibitor.
	Inhibitors are present in the reaction.	Ensure the presence of an inhibitor: 1. Create a serial dilution of your sample. 2. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, high concentrations yield higher-than-expected C_t values. (High concentration means more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template.
	The baseline and/or threshold was improperly set.	See your real-time PCR system user guide for procedures on setting the baseline and threshold: • Switch from an automatic baseline to a manual baseline, or from a manual baseline to an automatic baseline. • Lower the threshold value to within the appropriate range.
	The reverse transcription failed.	• Check the RNA integrity and concentration. • Check for RNase activity. • Follow the recommended thermal profile. • Repeat the reverse transcription using new reagents.

Observation	Possible cause	Recommended action
Amplification curve shows samples targeted by the same assay that have differently shaped curves	The baseline was set improperly.	See your real-time PCR system user guide for procedures on setting the baseline: • Switch from an automatic baseline to a manual baseline, or from a manual baseline to an automatic baseline. • Increase the upper or lower value of the baseline range.
	The sample quality was poor.	1. Perform a quality check on the sample. 2. If needed, re-extract the sample.
	There were different concentrations caused by imprecise pipetting.	Follow accurate pipetting practices.
	The reagents or equipment are contaminated.	Be sure that your workspace and equipment are cleaned properly.

Observation	Possible cause	Recommended action
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. Go to **ncbi.nlm.nih.gov/unigene**. • Confirm the results. – Rerun the sample using the same assay. – 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.	Check your pipetting equipment and/or technique.
	Incorrect dyes were selected for each target.	Check the dyes selected for each target, 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.
There was a simultaneous increase in fluorescence from both the passive reference dye (ROX™ dye) and the reporter dyes	The sample evaporated.	Check the seal of the adhesive film for leaks.
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.

Observation	Possible cause	Recommended action
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 20.
	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.
There is a noisy signal above the threshold	The sample evaporated.	Check the seal of the adhesive film for leaks.
	The well is empty because of inaccurate pipetting.	• Check the calibration of the pipettes. • Pipet at least 5 µL of sample.
	The well is assigned a sample or target in the plate document or experiment, but the well is empty.	• Be sure that your plate document or experiment is set up correctly. • Exclude the well and reanalyze the data.
The baseline is variable	The dried-down assays on the TaqMan® Array Plate were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Use the relative threshold algorithm (C_{rt}). C_{rt} may correct for a variable baseline.
		Use the Relative Quantification application, available on Connect. The Relative Quantification application uses C_{rt} if your instrument software does not have the relative threshold algorithm.

B

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. CO01971 0612), available from **thermofisher.com**.

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

TaqMan® Gene Expression Assays chemistry overview

TaqMan® MGB probes

TaqMan® MGB probes contain:

- A reporter dye (for example, FAM™) 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.

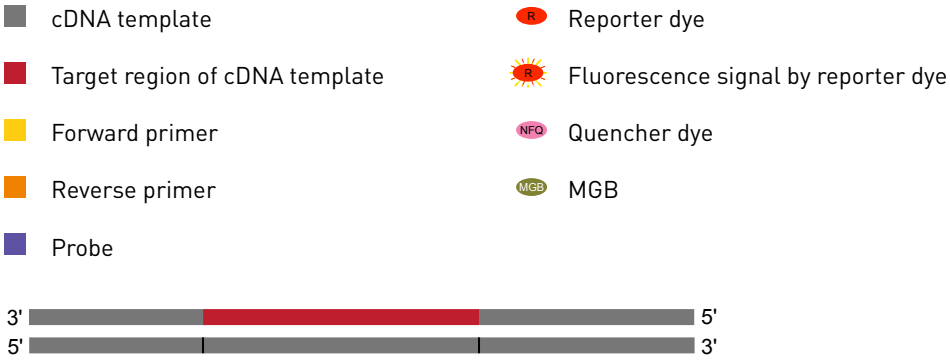


Figure 1 cDNA synthesis product

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.

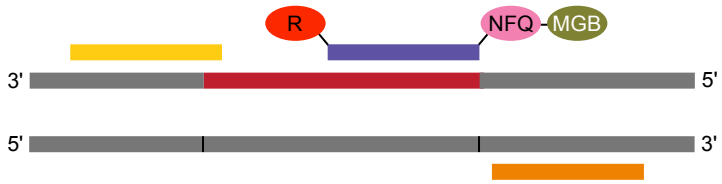


Figure 2 Annealing of probes and primers to cDNA strands

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.

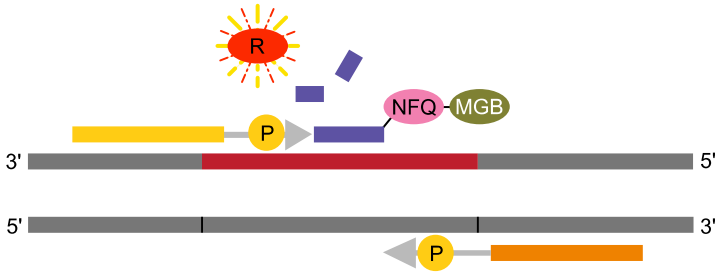


Figure 3 Initial polymerization and cleavage of reporter dye

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.

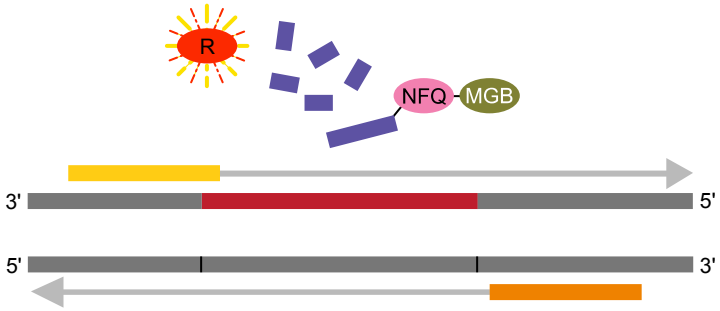


Figure 4 Completion of polymerization

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 will not affect the 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, change the thermal cycling conditions before starting the run. A longer enzyme activation time can help to denature double-stranded genomic DNA when genomic DNA is used.

Algorithms for data analysis

Table 6 Algorithm recommendations for TaqMan® Array Plates

Algorithm	Recommendation
Relative threshold (C_{rt})	• Recommended for data analysis. • Use to troubleshoot unexpected results. • Use to correct a variable baseline, which can be due to dried-down assays on the plate being reconstituted at different rates.

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

Thermal protocols

The thermal protocol settings depend on:

- The real-time PCR instrument
- Whether the Master Mix requires fast or standard cycling mode based on its chemistry
- Whether the Master Mix contains UNG

The thermal protocols in "Set up and run the real-time PCR instrument" on page 12 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 7 TaqMan® Gene Expression Master Mix or TaqMan® Universal Master Mix II, with UNG

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 8 TaqMan® Universal Master Mix II, no UNG

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 9 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG

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	

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.

C

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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

Documentation and support

Related documentation

Document	Pub. No.
TaqMan® Gene Expression Assays Quick Reference—384-well Plates	100085359
Introduction to Gene Expression Getting Started Guide	4454239
TaqMan® PreAmp Master Mix User Guide	4384557
TaqMan® PreAmp Master Mix Quick Reference	4384556
Understanding Your Shipment For detailed information about the Assay Information File (AIF)	MAN0017153
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems User Guide	MAN0018045
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Design and Analysis Desktop Software User Guide	MAN0010408
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ 6 and 7 Flex Real-Time PCR System 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

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- Product documentation
  - 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.