TaqMan® Pri-miRNA Assays

USER GUIDE

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Revision E

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, CA 94566
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. 4427719

Revision	Date	Description
E	16 July 2019	• Updated kit contents to remove CD and add file downloads. • Added new instruments, Master Mixes, and other applicable products. • Added instructions to dilute 60X assays. • Updated the order that components are added to the PCR Reaction Mix. • Updated for general style, formatting, and branding.
D	July 2010	Baseline for this revision history.

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Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Applied Biosystems™ TaqMan® Pri-miRNA Assay enables quantitative expression studies of primary microRNA (pri-miRNA) transcripts in human, mouse, and rat species using reverse transcription and real-time PCR (RT-PCR). The assays are predesigned, preformulated primer and probe sets.

Each of the assays is named after a Sanger miRBase stem-loop ID. The assay ID consisted of a prefix (indicating which species to which the assay is designed), a unique numeric string, and the suffix "_pri".

Assay ID prefix	Species
Hs	Homo *sapiens*
Mm	Mus *musculus*
Rn	Rattus *norvegicus*

Contents and storage

Table 1 TaqMan® Pri-miRNA Assays (made-to-order)

Size	Cat. No.	Concentration	Number of 20-µL reactions	Storage[1]
Small	4427012	20×	360	–25°C to –15°C. Protect from light.
Medium	4427013	20×	750	
Large	4427014	60×	2900	

[1] See label for expiration date.

Table 2 Contents of each assay

Item	Stock concentration	Final concentration
Unlabeled primers (2 each)	20× (18 µM)	1× (900 nM)
6-FAM™ dye-labeled TaqMan® MGB Probe	20× (5 µM)	1× (250 nM)

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

- Assay information files (AIFs)
- User Instruction Documents (Protocols, User Guides, and Quick Reference Cards)
- Certificates of Analysis
- Safety Data Sheets

For detailed information about the shipment and assay information files (AIF), see *Understanding Your Shipment* (Pub. No. MAN0017153).

Required materials not supplied

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

Table 3 Recommended products for isolation of RNA

Item	Source
Kits for RNA isolation	thermofisher.com/rnaisolation

Table 4 Recommended products for preparation of cDNA

Items	Source
High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor	4374966
High-Capacity RNA-to-cDNA™ Kit	4387406
TaqMan® RNA-to-C_T™ 1-Step Kit	4392653

Table 5 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 6 Other materials and equipment required for the workflow

Item	Source
Real-time PCR instrument, one of the following:	
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	Contact your local sales office
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
StepOne™ or StepOnePlus™ Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
7500/7500 Fast Real-Time PCR System	
Software	
Relative Quantification application	**thermofisher.com/cloud**
Standard Curve application	
ExpressionSuite™ Software[1]	**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
Centrifuge, with adapter for 96-well or 384-well plates	MLS
Microcentrifuge	MLS
Vortex mixer	MLS
(Optional) Eppendorf™ MixMate™ (shaker)	Fisher Scientific™ 21-379-00
Pipettes	MLS
Tubes, plates, and film	**thermofisher.com/plastics**
Disposable gloves	MLS
Pipette tips, aerosol resistant	MLS
Reagents	
TURBO DNA-*free*™ Kit	AM1907
Nuclease-free Water (not DEPC-Treated)	AM9930

Item	Source
Nuclease-free Water	AM9938
TE, pH 8.0	AM9849

[1] Can automatically define the baseline. Files from a QuantStudio™ 3 or 5 System are not compatible.

Workflow

Start with cDNA templates prepared from RNA samples (page 9)

▼

Prepare the PCR Reaction Mix (page 11)

▼

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

▼

Analyze the results (page 13)

2 Guidelines for preparation of cDNA

Guidelines for isolation of high–quality RNA

- See Table 3 on page 6 for recommended RNA isolation kits.
- The assay detects genomic DNA. If your RNA purification method does not include DNase treatment, treat the purified RNA with the TURBO DNA-*free*™ Kit using the standard protocol.

Guidelines for cDNA template preparation

- See Table 4 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
  - Nondegraded total RNA (not applicable for double-stranded templates)

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

3 Perform real-time PCR

Procedural guidelines for performing real-time PCR

- Follow best-practices when preparing or performing PCR (see page 26).
- Use the same amount of cDNA in each reaction.
- Prepare the real-time PCR reactions in an area free of artificial templates and siRNA transfections. High-copy-number templates can easily contaminate the real-time PCR reactions.
- Configure run documents according to the instructions provided in the real-time PCR instrument user documents.
- Protect the assays from light and store as indicated until ready for use. Excessive exposure to light can negatively affect the fluorescent probes of the assays.
- Run four technical replicates to identify outliers.

Before you begin

- Determine the total number of PCR reactions needed, including replicates, for each sample. Include an endogenous control and a no template control for each assay.

  Note: We recommend four replicates for each assay.

- Thaw the assays on ice, then vortex and briefly centrifuge to resuspend.

(60X assays only) Dilute the assays

Dilute 60X assays to 20X working stocks with TE, pH 8.0, then divide the solutions into smaller aliquots to minimize freeze-thaw cycles. The size of the aliquots depends upon the number of PCR reactions. An example dilution is shown in the following table.

1. Gently vortex the tube of 60X assay, then centrifuge briefly to spin down the contents and eliminate air bubbles.
2. In a 1.5-mL microcentrifuge tube, dilute sufficient amounts of 60X assay for the required number of reactions.

Component	Volume
TaqMan® Pri-miRNA Assays (60X)	40 µL
TE, pH 8.0 (1X)	80 µL
Total aliquot volume	**120 µL**

3. Store aliquots at –20°C until use.

Prepare the PCR Reaction Mix

Thaw the cDNA samples on ice. Resuspend the cDNA samples by inverting the tube, then gently vortexing.

1. Mix the Master Mix thoroughly but gently.
2. Combine the PCR Reaction Mix and assays in an appropriately-sized microcentrifuge tube according to the following table.

Component	Volume for 1 reaction	
	Standard 96–well or 48–well Plates	384–well Plate or 96–well Fast Plate
Master Mix (2X)[1,2]	10 µL	5 µL
TaqMan® Pri-miRNA Assay (20X)	1 µL	0.5 µL
Nuclease-free Water[3]	5 µL	2.5 µL
PCR Reaction Mix volume	**16 µL**	**8 µL**

[1] Recommended: TaqMan® Fast Advanced Master Mix

[2] (*Optional*) If you add AmpErase™ UNG (uracil-N-glycosylase), the final concentration must be 0.01U/ µL. Reduce the volume of water in the PCR reaction mix to compensate for additional volume from the UNG.

[3] Adjust the volume of Nuclease-free Water to for a larger volume of cDNA

3. Vortex to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
4. Transfer the appropriate volume of PCR Reaction Mix to each well of an optitcal reaction plate.

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

   Note: Adjust the volume of nuclease-free water in the PCR reaction mix for a larger volume of cDNA.

   IMPORTANT! For optimal results when using TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG, prepare the plate on ice. Run the plate within 2 hours of preparation, or store the plate at 2–8°C for up to 24 hours.

6. Seal the plate with optical adhesive film, then vortex briefly to mix the contents.

7. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

Set up and run the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

Note: The instrument must be configured with the block appropriate for the plate type.

1. Select the cycling mode appropriate for the Master Mix.

   IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.

2. Set up the thermal protocol for your instrument.

   See "Thermal protocols" on page 24 for the thermal protocols for other Master Mixes.

   Table 7 TaqMan® Fast Advanced Master Mix (StepOne™, StepOnePlus™, ViiA™ 7, and QuantStudio™ systems with fast cycling mode)

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

[1] Optional, for optimal UNG activity.

   Table 8 TaqMan® Fast Advanced Master Mix (7500 and 7500 Fast systems with fast cycling mode)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

[1] Optional, for optimal UNG activity.

3. Set the reaction volume appropriate for the reaction plate.
   - 96 or 48-well Standard (0.2-mL) plates: **20 µL**
   - 96-well Fast (0.1-mL) Plate and 384–well plates: **10 µL**

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

5. Start the run.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your instrument. Use the absolute or relative quantification ($\Delta\Delta C_t$) methods to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values.
  - Remove outliers from the analysis.
- In the well table or results table, view the C_t values for each well and for each replicate group.

Perform additional analysis using any of the following software:

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

[1] Can automatically define the baseline. Files from a QuantStudio™ 3 or 5 System are not compatible.

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

Algorithms for data analysis

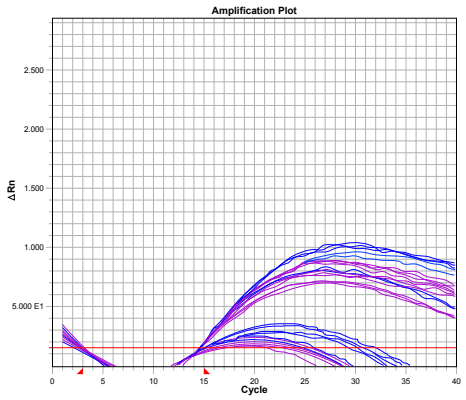
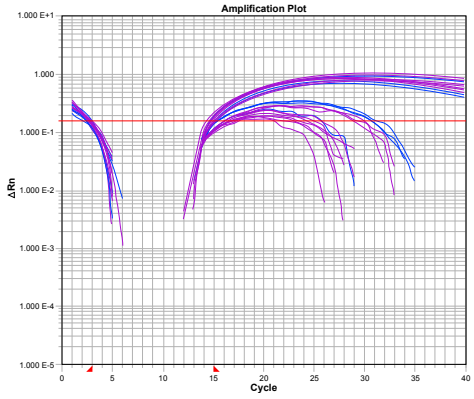
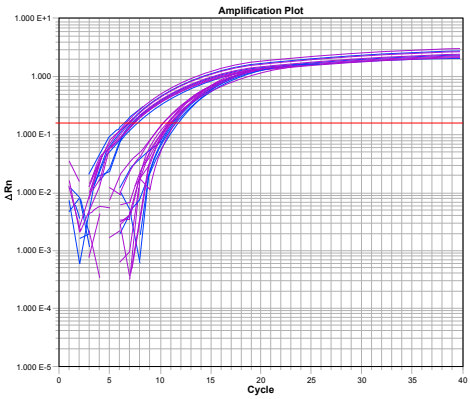
Table 9 Algorithm recommendations for single–tube assays

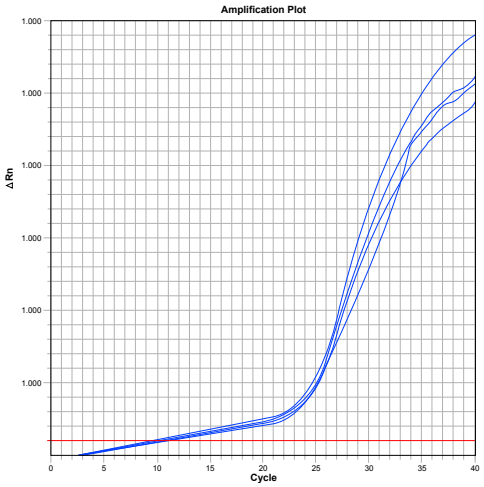
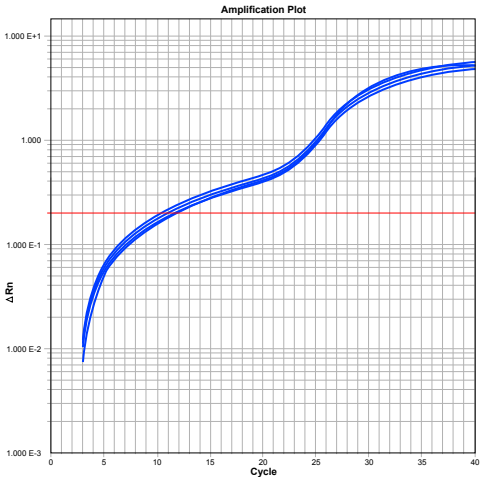
Algorithm	Recommendation
Threshold (C_t)	Recommended.
Relative threshold (C_{rt})	*(Optional)* Use for troubleshooting abnormal or unexpected results.

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

A

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.

Observation	Possible cause	Recommended action
Amplification curve shows a rising baseline Linear view:  Log view:  	There is interaction between the primer and probe.	Adjust the threshold manually. Select another assay from the same gene, if available.

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, the assay, 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 containing UNG or add UNG separately, then repeat the experiment. • Run no-RT controls to rule out genomic DNA contamination. • Treat the sample with DNase. • Design an assay that spans an exon-exon boundary if genomic DNA contamination is suspected.
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 22.
	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.

Observation	Possible cause	Recommended action
The C_t value is lower than expected	Contamination occurred.	• Run no-RT control to confirm that there was genomic DNA (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.
	The cDNA template or the amplicon is contaminated.	Follow established PCR good laboratory practices.
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.

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® Pri-miRNA 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

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

During the PCR, the TaqMan® MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites.

When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Förster–type energy transfer.

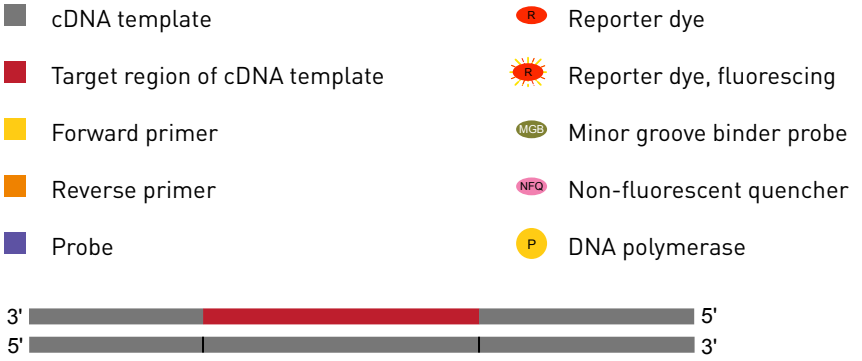


Figure 1 cDNA synthesis product

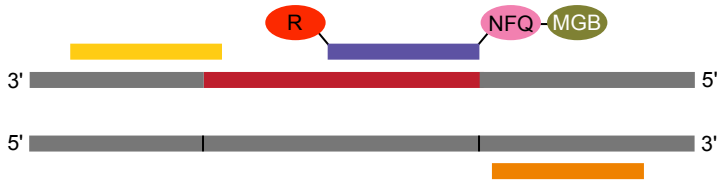


Figure 2 Denature and anneal

The DNA polymerase cleaves only probes that hybridize to the target. Cleavage separates the reporter dye from the quencher dye. This results in increased fluorescence by the reporter. The increase in fluorescence occurs only if the target sequence is complementary to the probe and amplified during PCR. Because of these requirements, nonspecific amplification is not detected.

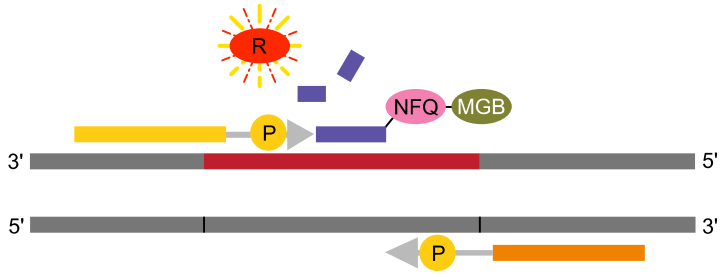


Figure 3 Cleavage

Polymerization of the strand continues. However, no extension of the probe occurs during PCR because the 3' end of the probe is blocked.

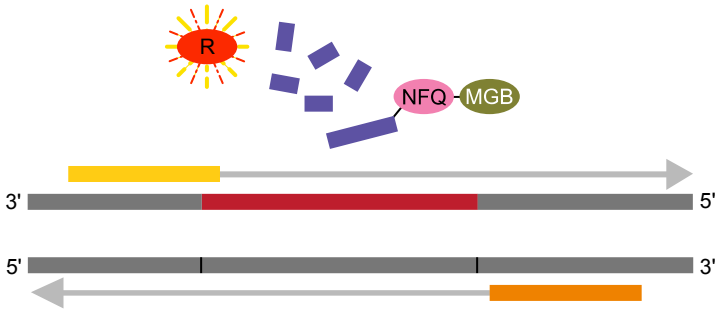


Figure 4 Completion of polymerization

Design of the TaqMan® Pri-miRNA Assay

TaqMan® Pri-miRNA Assays are designed with the same algorithms as TaqMan® Gene Expression Assays. The assay targets sequences near the stem-loops that are identified in the Sanger miRBase sequence repository. Each assay targets areas within 500 nucleotides on either side of the stem-loop. In a small portion of cases, stem-loop sequences are sufficiently close so as to prevent design to the intervening sequence. In these cases, we recommend using the nearest available assay (Figure 5).

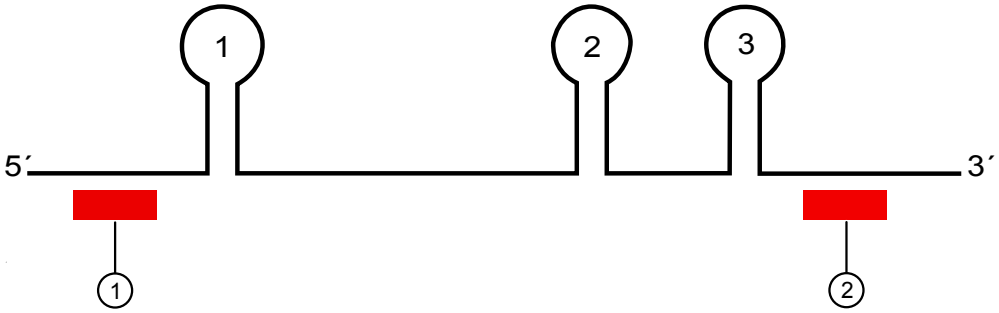


Figure 5 TaqMan® Pri-miRNA Assay alignments
① Stem-loop 1: detected by assay 1
② Stem-loops 2 and 3: detected by assay 2

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® Pri-miRNA Assays.

IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.

Table 10 TaqMan® Gene Expression Master Mix or TaqMan® Universal Master Mix II, with UNG (any compatible instrument)

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

[1] For optimal UNG activity.

Table 11 TaqMan® Universal Master Mix II, no UNG (any compatible instrument)

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

Table 12 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (StepOne™, StepOnePlus™, ViiA™ 7, or QuantStudio™ system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

Table 13 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (7500 or 7500 Fast system)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

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.

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® Pri-miRNA Assays Quick Reference Guide	4427720
Introduction to Gene Expression Getting Started Guide	4454239
Understanding Your Shipment	MAN0017153
Custom TaqMan® Assays Design and Ordering Guide	4367671
Custom TaqMan® Small RNA Assays Design and Ordering Guide	4412550
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 Pro and 7 Pro Real-Time PCR Systems	
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems User Guide	MAN0018045
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
StepOne™ or StepOnePlus™ Real-Time PCR System	
StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide	4376782
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785

Document	Pub. No.
7500/7500 Fast Real-Time PCR System	
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments	4387783

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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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