TaqMan™ Small RNA Assays

USER GUIDE

TaqMan™ MicroRNA Assays, Custom TaqMan™ Small RNA Assays, and TaqMan™ siRNA Assays

Publication Number 4364031

Revision J

ThermoFisher SCIENTIFIC

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

Revision history: 4364031 J (English)

Revision	Date	Description
J	26 June 2024	A statement about 1,000 reactions was added ("Prepare the RT Reaction Mix" on page 13).
H	10 December 2019	Removed troubleshooting and replaced with link to FAQs.
G	23 July 2019	• Corrected volumes for PCR Reaction Mix and amount of cDNA template to add. • Added QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System. • Removed the instruction to add a compression pad to the real-time PCR plate. • Added a recommended starting point for the threshold value when analyzing the data. • Added information about UNG. • Added information about secondary analysis software. • Removed recommendation to use the Megaplex™ Assay Performance File in troubleshooting non-specific interactions between primers.
F	12 February 2019	• Added new instruments, Master Mixes, and other applicable products. • Added thermal cycling protocols for all compatible Master Mixes. • Updated options for secondary analysis software. • Added troubleshooting information. • Updated for general style, formatting, and branding.
E	1 January 2011	Baseline for this revision history.

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

Contents

1 Product information

Product description

Applied Biosystems™ TaqMan™ Small RNA Assays are primer and probe sets designed to detect and quantify mature microRNAs (miRNAs), small interfering RNAs (siRNAs), and other small RNAs. The assays can detect and quantify small RNA in 1 to 10 ng of total RNA with a dynamic range of greater than six logs. When used for microRNA analysis, the assays can discriminate mature miRNA sequences from their precursors.

Note: In this user guide, "small RNA" refers to miRNA, siRNA, or other small RNAs that are less than 200 bases in length.

Predesigned and custom TaqMan™ Small RNA Assays are available for a variety of small RNA classes.

- TaqMan™ MicroRNA Assays
  - Predesigned assays for the majority of content found in the miRBase miRNA sequence repository.
  - Ideal for targeted quantification, screening, and validation of miRNA profiling results.
- TaqMan™ siRNA Assays for *Silencer*™ Select siRNAs
  - Predesigned assays for the quantification of *Silencer*™ Select siRNAs.
  - Ideal for assessing siRNA transfection efficiency, half-life, and bio-distribution.
- TaqMan™ Small RNA Controls
  - Predesigned assays for small, non-coding RNAs unrelated to miRNAs used to normalize for differences in sample RNA.
  - TaqMan™ Small RNA Controls are available for a number of species.

  For more information on using TaqMan™ control assays, see "Select a TaqMan™ Small RNA Control" on page 6.
- Custom TaqMan™ Small RNA Assays
  - Custom assays designed for any small RNA sequence from 17–200 nucleotides in length.
  - We provide ready-to-use Custom TaqMan™ Small RNA Assays based on customer–supplied target sequences for any organism, with optimized primers and probe.

Overview of TaqMan™ Small RNA Assays

TaqMan™ Small RNA Assays use a stem-looped primer for reverse transcription and a sequence-specific assay to accurately detect mature miRNAs, siRNAs, and other small RNAs.

Each assay includes two tubes.

- A tube of small RNA–specific stem–looped RT Primer.
- A tube containing a mix of small RNA–specific forward PCR Primer, small RNA–specific reverse PCR Primer, and small RNA–specific TaqMan™ MGB probe.

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

TaqMan™ Small RNA Controls

We recommend using TaqMan™ Small RNA Controls with TaqMan™ Small RNA Assays. When quantifying small RNA gene expression levels, variation in the amount of starting material, sample collection, RNA preparation and quality, and reverse transcription (RT) efficiency can contribute to quantification errors. Normalization to endogenous control genes is currently the most accurate method to correct for potential RNA input or RT efficiency biases.

An ideal endogenous control generally shows gene expression that is relatively constant and highly abundant across tissues and cell types. You need to verify the chosen endogenous control or set of controls for the target cell, tissue, or treatment because no single control can act as a universal endogenous control for all experimental conditions.

To view a complete list of available controls, use the assay search tool at **thermofisher.com/taqmanmirna**.

Contents and storage

Table 1 TaqMan™ MicroRNA Assays

Cat. No.	Number of 20–µL reactions	Amount and concentration		Storage
		RT Primer	Assay	
Inventoried predesigned assays				
4427975 (Small)	50 RT 150 PCR	150 µL (5×)	150 µL (20×)	–25°C to –15°C
Made–to–order predesigned assays				
4440888 (Large)	2900 RT 2900 PCR	725 µL (60×)	967 µL (60×)	–25°C to –15°C
4440887 (Medium)	750 RT 750 PCR	575 µL (20×)	750 µL (20×)	
4440886 (Small)	50 RT 150 PCR	150 µL (5×)	150 µL (20×)	
4440885 (Extra small)	25 RT 75 PCR	75 µL (5×)	75 µL (20×)	

Table 2 Custom TaqMan™ Small RNA Assays

Cat. No.	Number of 20–µL reactions	Amount and concentration		Storage
		RT Primer	Assay	
4398989 (Large)	2900 RT 2900 PCR	725 µL (60×)	967µL (60×)	–25°C to –15°C
4398988 (Medium)	750 RT 750 PCR	575 µL (20×)	750 µL (20×)	
4398987 (Small)	50 RT 150 PCR	150 µL (5×)	150 µL (20×)	
4440418 (Extra small)	25 RT 75 PCR	75 µL (5×)	75 µL (20×)	

Table 3 TaqMan™ siRNA Assays (predesigned)

Cat. No.	Number of 20–µL reactions	Amount and concentration		Storage
		RT Primer	Assay	
4440880 (Large)	2900 RT 2900 PCR	725 µL (60×)	967 µL (60×)	–25°C to –15°C
4440879 (Medium)	750 RT 750 PCR	575 µL (20×)	750 µL (20×)	
4440878 (Small)	50 RT 150 PCR	150 µL (5×)	150 µL (20×)	
4440877 (Extra small)	25 RT 75 PCR	75 µL (5×)	75 µL (20×)	

Order TaqMan™ Small RNA Assays

Order predesigned and custom assays for miRNAs, siRNAs, and other small RNAs at **thermofisher.com/taqmanmirna**. There are tools to help select inventoried assays or design custom assays for an unlisted small RNA.

Order Custom TaqMan™ Small RNA Assays or predesigned TaqMan™ siRNA Assys for *Silencer*™ Select siRNAs at **Silencer Select siRNAs**.

For information on designing custom assays, see *Custom TaqMan™ Small RNA Assays Design and Ordering Guide* (Pub. No. 4412550).

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 4 Recommended products for isolation of RNA

Sample type	Item	Source
Tissue samples	*mir*Vana™ miRNA Isolation Kit, with phenol	AM1560
	*mir*Vana™ miRNA Isolation Kit, without phenol	AM1561
	*mir*Vana™ PARIS™ RNA and Native Protein Purification Kit	AM1556
	MagMAX™ *mir*Vana™ Total RNA Isolation Kit	A27828
	TRI Reagent™ Solution	AM9738
	RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
Cell samples	TaqMan™ MicroRNA Cells-to-C_T Kit	4391848
	Cells-to-C_T™ Stop Solution, 1 mL	4402960

Table 4 Recommended products for isolation of RNA *(continued)*

Sample type	Item	Source
Cell samples	Cells-to-C_T™ Bulk Lysis Reagents	4391851C
	MagMAX™ *mir*Vana™ Total RNA Isolation Kit	A27828
Liquid samples	MagMAX™ *mir*Vana™ Total RNA Isolation Kit	A27828

Table 5 Recommended product for preparation of cDNA

Item	Source
TaqMan™ MicroRNA Reverse Transcription Kit[1]	4366596

[1] TaqMan™ Small RNA Assays are optimized for the MuLV Reverse Transcriptase contained in the TaqMan™ MicroRNA Reverse Transcription Kit. Performance with other RT enzymes cannot be guaranteed.

Table 6 PCR Master Mixes

Item	Source
TaqMan™ Fast Advanced Master Mix	4444558
TaqMan™ Universal Master Mix II, no UNG	4440043
TaqMan™ Universal Master Mix II, with UNG	4440042
TaqMan™ Universal PCR Master Mix, no AmpErase™ UNG	4364341
TaqMan™ Universal PCR Master Mix	4304437

Table 7 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	

Item	Source
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 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*™ KitDNase	AM1907
TE, pH 8.0	AM9849

Workflow

Start with total RNA

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Prepare the RT Reaction Mix (page 13)

▼ ▼

Prepare RT reaction with double–stranded small RNA (page 14)

Prepare RT reaction with single–stranded small RNA (page 14)

▼

Perform reverse transcription (page 15)

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Prepare the PCR Reaction Mix (page 17)

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Prepare the PCR reaction plate (page 17)

▼

Set up and run the real–time PCR instrument (page 18)

▼

Analyze the results (page 19)

2 Perform reverse transcription

Guidelines for isolation of high–quality RNA

- See Table 4 on page 8 for recommended RNA isolation kits.
- Prepare samples using a method that preserves small RNAs.
- To prevent the loss of the longer control transcripts (such as snoRNAs), size fractionation is *not* recommended.

Guidelines for RNA input

- Use 1–10 ng of total RNA per 15–µL RT reaction.
- For optimal reverse transcription, input RNA should have the following characteristics:
  - Free of inhibitors of reverse transcription (RT) and PCR.
  - Dissolved in PCR-compatible buffer.
  - Free of RNase activity.
  - Nondenatured total RNA (not applicable for double-stranded templates).

  IMPORTANT! Do not denature the total RNA.
- Use 10 to 10,000 cells per sample for the TaqMan™ MicroRNA Cells-to-C_T Kit.
- When working with double-stranded template such as siRNAs and miRNA mimics, denature the siRNA template with sequence-specific RT Primer before performing the reverse transcription.
- Prepare the RT reactions in an area free of artificial templates, amplified material, and siRNA transfections. High-copy-number templates can easily contaminate the reactions.
- When working with siRNA use total RNA from non-transfected cells as a control in *in vitro* and *in vivo* studies. The amount of input total RNA for optimal detection depends on the transfection protocol used.

Note: When testing for more than 10 targets, it might be more efficient to create primer pools. For details see *Protocol for Creating Custom RT and Preamplification Pools Using TaqMan™ MicroRNA Assays* (Pub. No. 4465407).

IMPORTANT! TaqMan™ MicroRNA Assays and TaqMan™ Small RNA Assays are optimized for the MuLV Reverse Transcriptase contained in the TaqMan™ MicroRNA Reverse Transcription Kit. Assay performance with other reverse transcriptase enzymes cannot be guaranteed.

Before you begin

- Thaw components of the reverse transcription kit on ice.
- Thaw the RT Primers on ice, vortex briefly, then centrifuge briefly to collect the contents at the bottom of the tube.
- (*Large assays only*) Dilute the 20× or 60× RT Primer to a 5× working solution using 0.1× TE Buffer. Store for up to one year at –25°C to –15°C.

Prepare the RT Reaction Mix

1. In an appropriately-sized microcentrifuge tube, prepare RT Reaction Mix according to the following table.

   For single miRNA/small RNA assays requiring 1 µl/rxn, the kit (4366597) contains enough RT enzyme for 1,000 reactions. For arrays requiring 3 µl/rxn, the kit (4366597) contains enough RT enzyme for 1,000 reactions.

Component	Volume (1 reaction)	Volume (10 reactions)[1]
100mM dNTPs (with dTTP)	0.15 µL	1.65 µL
MultiScribe™ Reverse Transcriptase, 50 U/µL	1.00 µL	11.00 µL
10× Reverse Transcription Buffer	1.50 µL	16.50 µL
RNase Inhibitor, 20 U/µL	0.19 µL	2.09 µL
Nuclease-free Water	4.16 µL	45.76 µL
Total RT Reaction Mix volume	**7.00 µL**	**77.00 µL**

[1] Includes 10% overage.

2. Invert to mix, then centrifuge briefly to collect the contents at the bottom of the tube.

Place the RT Reaction Mix on ice. Proceed immediately to “Prepare the RT reaction” on page 14.

Prepare the RT reaction

Use one of the following procedures.

Prepare RT reaction with double–stranded small RNA

Silencer Select miRNAs are double–stranded miRNA–mimicking molecules.

1. Combine 3 µL of 5× RT Primer and 5 µL of double-stranded template in a reaction tube or in each well of a reaction plate.
   5 µL should contain 1–10 ng of double–stranded template.
2. Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube or wells.
3. Incubate at 85°C for 5 minutes.
4. Incubate at 60°C for 5 minutes, then place on ice.
5. Add 7 µL of RT Reaction Mix to each reaction tube or well.
   RT Reaction Mix was prepared in “Prepare the PCR Reaction Mix” on page 17.
6. Seal the tube or reaction plate.
7. Centrifuge briefly to collect the contents at the bottom of the tubes or wells.

Place on ice and proceed immediately to “Perform reverse transcription” on page 15.

Prepare RT reaction with single–stranded small RNA

1. Combine 7 µL of RT Reaction Mix and 5 µL of total RNA in a reaction tube or in each well of a reaction plate.
   5 µL should contain 1–10 ng of total RNA.
   RT Reaction Mix was prepared in “Prepare the RT Reaction Mix” on page 13.
2. Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tubes or wells.
3. Add 3 µL of 5× RT Primer to each reaction tube or each well of a reaction plate.
4. Seal the tubes or reaction plate.
5. Centrifuge briefly to collect the contents at the bottom of the tubes or wells.

Place on ice and proceed immediately to “Perform reverse transcription” on page 15.

Perform reverse transcription

Place the reaction plate or tubes into a thermal cycler, then incubate using standard cycling, a reaction volume of 15.0 µL, and the following settings.

Step	Temperature	Time
Reverse transcription	16°C	30 minutes
	42°C	30 minutes
Stop reaction	85°C	5 minutes
Hold	4°C	Hold

The RT reaction product can be stored at –25 to –15°C for up to one week.

3 Perform PCR amplification

Procedural guidelines for performing real–time PCR

- Follow best practices when preparing or performing PCR. See “Good laboratory practices for PCR and RT-PCR” on page 25.
- Prepare the PCR reactions in an area free of artificial templates and siRNA transfections. High-copy-number templates can easily contaminate the real-time PCR reactions.
- Protect the assays from light and store in a freezer. Excessive exposure to light might affect the fluorescent probes.
- Prepare the PCR reaction mix before transferring it to the reaction plate for thermal cycling and fluorescence analysis.
- Perform three replicates of each reaction.
- Include the following reactions:
  - A small RNA assay for each cDNA sample.
  - Endogenous control assays for each cDNA sample.
  - No–template controls (NTCs) for each assay on the plate.

Note: Use of NTC reactions to evaluate background signal is strongly recommended.

Before you begin

- (*For large assays*) Dilute 60× assays to 20× working solutions before use.
- Thaw assays (20×) and cDNA templates on ice, vortex gently, then centrifuge briefly to bring the contents to the bottom of the tube.

Prepare the PCR Reaction Mix

1. Mix the PCR Master Mix by gently swirling the bottle.

   See "Required materials not supplied" on page 8 for a list of compatible PCR Master Mixes.

2. Prepare the PCR Reaction Mix in appropriately-sized microcentrifuge tubes, according to the following table.

Component	Volume per reaction[1]	
	384–well or 96–well fast (0.1mL) plate	96–well standard (0.2 mL) plate
TaqMan™ Small RNA Assay (20X)	0.50 µL	1.00 µL
PCR Master Mix	5.00 µL	10.00 µL
Nuclease–free water	3.84 µL	7.67 µL
Total PCR Reaction Mix volume	**9.34 µL**	**18.67 µL**

[1] Add 10% overage to account for pipetting loss.

Note: The volume of nuclease-free water can be adjusted if a smaller volume of cDNA template is added. The recommended volume of cDNA template is the maximum volume of cDNA template that should be added, due to the required dilution of the RT Primer in the final PCR (see "Prepare the PCR reaction plate" on page 17).

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

Prepare the PCR reaction plate

1. Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.

   - 384–well or 96–well fast (0.1 mL) plate: 9.34 µL
   - 96–well standard (0.2 mL) plate: 18.67µL

   The PCR Reaction Mix was prepared in "Prepare the PCR Reaction Mix" on page 17.

2. Add cDNA template, or nuclease–free water for NTC, to each well of an optical reaction plate.

   - 384–well or 96–well fast (0.1 mL) plate: 0.67 µL
   - 96–well standard (0.2 mL) plate: 1.33 µL

   Note: This is the maximum volume of cDNA template that can be added to the PCR reaction.

   Adjust the volume of nuclease–free water in the PCR Reaction Mix for a smaller volume of cDNA template (see "Prepare the PCR Reaction Mix" on page 17).

3. Seal the plate with optical adhesive film, then centrifuge the plate briefly to bring the PCR Reaction Mix to the bottoms 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.

Table 8 TaqMan™ Fast Advanced Master Mix (StepOne™, StepOnePlus™, ViiA™ 7, and QuantStudio™ systems with fast cycling mode)

Step	Temperature	Time	Cycles
(*Optional*) UNG activation	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

Table 9 TaqMan™ Fast Advanced Master Mix (7500 and 7500 Fast systems with fast cycling mode)

Step	Temperature	Time	Cycles
(*Optional*) UNG activation	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

Table 10 TaqMan™ Universal Master Mix II, no UNG, TaqMan™ Universal Master Mix II, with UNG, TaqMan™ Universal PCR Master Mix, no AmpErase™ UNG, or TaqMan™ Universal PCR Master Mix (any compatible instrument with standard cycling mode)

Step	Temperature	Time	Cycles
(*Optional*) UNG activation	50°C	2 minutes	1
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	60 seconds	

3. Set the appropriate reaction volume.
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.
    A threshold value of 0.2 is a recommended starting point.
  - 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
DataAssist™ Software	thermofisher.com/dataassist

[1] Can automatically define the baseline.

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

Secondary analysis software

ExpressionSuite™ Software utilizes the comparative C_T ($\Delta\Delta C_T$) method to quantify relative gene expression across a large number of samples.

ExpressionSuite™ Software is available for download at thermofisher.com/expressionsuite.

DataAssist™ Software is a data analysis tool for sample comparison when using the comparative C_T ($\Delta\Delta C_T$) method for calculating relative quantitation of gene expression. The software uses a filtering procedure for outlier removal and various normalization methods based on lists of single or multiple genes. It provides relative quantification analysis of gene expression through a combination of statistical analysis and interactive visualization.

DataAssist™ Software is available for download at thermofisher.com/dataassist.

Algorithms for data analysis

Table 11 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**).

Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated with new information, guidance, and data.

- For troubleshooting information and FAQs for this product: **http://thermofisher.com/taqmansmallrnafaqs**
- To browse the database and search using keywords: **thermofisher.com/faqs**

B

Supplemental information

Overview of TaqMan™ Small RNA Assay chemistry

Reverse transcription

cDNA is reverse transcribed from total RNA using a small RNA–specific, stem–looped primer.

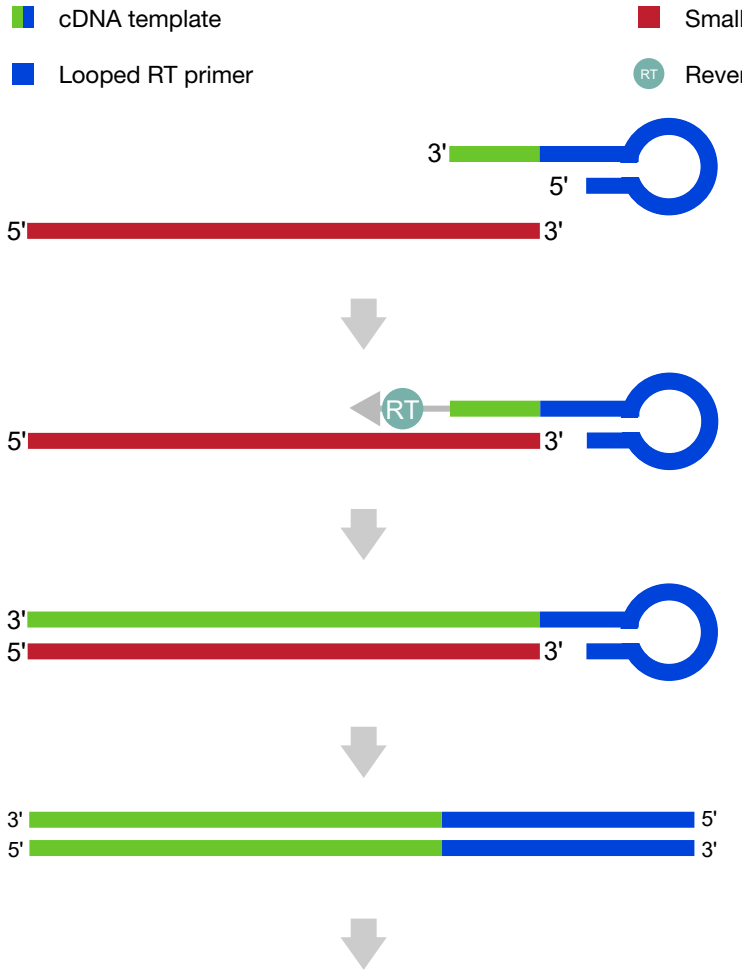

Figure 1 Stem loop primer

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™ Small RNA 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.

cDNA template
Forward primer
Probe
Reverse primer
Minor groove binder probe
Non-fluorescent quencher
Reporter dye
Fluorescing reporter dye
DNA polymerase

During the PCR, the forward and reverse primers anneal to complementary sequences along the denatured cDNA template strands.

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 and quencher dye suppresses the reporter fluorescence, primarily by Förster-type energy transfer.

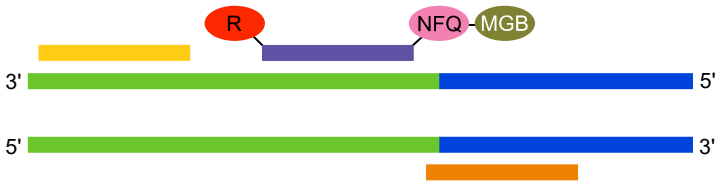

Figure 2 Annealing of probes and primers to cDNA strands or preamplified cDNA strands

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.

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.

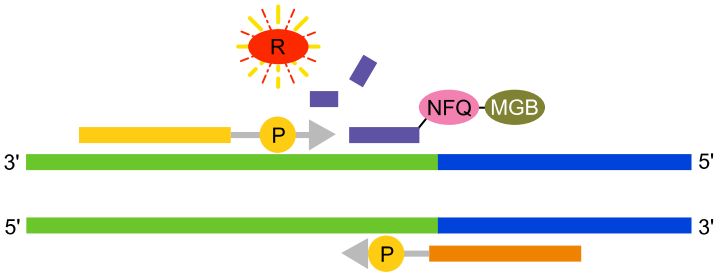

Figure 3 Initial polymerization and cleavage of reporter dye

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

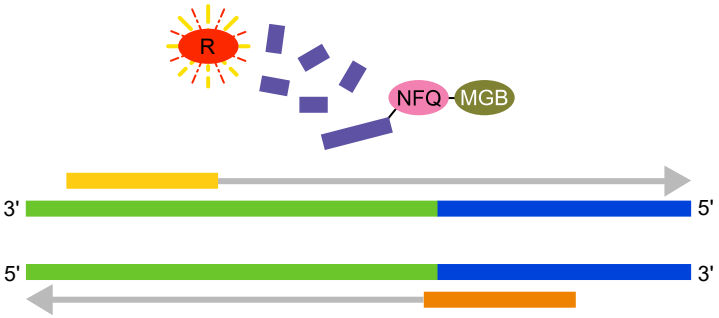

Figure 4 Completion of polymerization

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

Note: Use a PCR master mix without UNG if a preamplification step is required.

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.

The following items are recommended to enable 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! 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™ Small RNA Assay Quick Reference	4412551
Custom TaqMan™ Small RNA Assays Design and Ordering Guide	4412550
Introduction to Gene Expression Getting Started Guide	4454239
Custom Reverse Transcription Pools and Custom Preamplification Pools with TaqMan™ MicroRNA Assays User Bulletin	4465407
Understanding Your Shipment	MAN0017153

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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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