

TaqMan[®] PreAmp Master Mix

USER GUIDE

for use with TaqMan[®] Gene Expression Assays

Catalog Numbers 4391128, 4384267

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Revision D



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Revision	Date	Description
D	11 July 2018	<ul style="list-style-type: none">• Updated enzyme inactivation step in preamplification to be optional.• Updated guidelines and instructions for pooling TaqMan[®] Assays.• Updated volumes for preamplification reactions and PCR reactions in 96-well Standard (0.2-mL) Plates and 96-well Fast (0.1-mL) Plates.• Update storage temperature for preamplification products.• Changed recommended number of replicates for real-time PCR.• Added instructions to prepare PCR Reaction Mix for TaqMan[®] Array Plates.• Updated list of compatible real-time PCR systems and Master Mixes.• Added thermal cycling conditions to include all compatible Master Mixes and real-time PCR systems.• Corrected thermal cycling conditions and added ramp rates for TaqMan[®] Array Cards.• Used the term C_q to generically refer to cycle values.
C	1 February 2018	<ul style="list-style-type: none">• Added an enzyme inactivation step to the preamplification conditions.• Updated the list of compatible real-time PCR systems.• Rebranded document. Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
B	July 2010	Baseline for this revision history.

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Product information

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

Product description

Applied Biosystems™ TaqMan® PreAmp Master Mix enables unbiased, multiplex, preamplification of up to 100 specific cDNA targets from small quantities of cDNA (1–250 ng).

A pool of up to 100 TaqMan® Gene Expression Assays of interest are combined with cDNA and TaqMan® PreAmp Master Mix for preamplification PCR. The resulting preamplification product is then used for PCR using the corresponding TaqMan® Gene Expression Assays.

TaqMan® PreAmp Pools and Custom TaqMan® PreAmp Pools are pre-pooled sets of TaqMan® Gene Expression Assays available for downstream use with compatible TaqMan® Arrays.

Preamplification can also be performed prior to TaqMan® SNP Genotyping Assays.

TaqMan® PreAmp Master Mix is available alone (Cat. Nos. 4391128 and 4488593) or in combination with the TaqMan® Gene Expression Master Mix (Cat. No. 4384267). See “Contents and storage” on page 6.

The procedures in this document are for use with TaqMan® Gene Expression Assays in a single-tube format. For detailed information about other TaqMan® Gene Expression Assay formats or TaqMan® SNP Genotyping Assays, see the appropriate user guide (see “Related documentation” on page 34).

Contents and storage

Table 1 TaqMan® PreAmp Master Mix

Cat. No.	Number of reactions	Amount	Storage ^[1]
4391128	40	1 mL	2–8°C
4488593	200	5 mL	

^[1] See packaging for expiration date.

Table 2 TaqMan® PreAmp Master Mix Kit (Cat. No. 4384267)

Contents	Amount	Storage ^[1]
TaqMan® PreAmp Master Mix (2X)	1 mL ^[2]	2–8°C
TaqMan® Gene Expression Master Mix	5 mL	

^[1] See packaging for expiration date.

^[2] Sufficient for 40 reactions.

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 preparation of preamplification pools of TaqMan® Assays

Item	Source
TaqMan® Gene Expression Assays	thermofisher.com/taqmangeneexpression
<i>(Optional)</i> TaqMan® PreAmp Pool	Contact your local sales office.
<i>(Optional)</i> Custom TaqMan® PreAmp Pool	

Table 4 Recommended products for isolation of RNA

Item	Source
Kits for RNA isolation	thermofisher.com/rnaisolation

Table 5 Recommended products for preparation of cDNA

Item	Source
High-Capacity cDNA Reverse Transcription Kit	4368813
SuperScript™ VILO™ cDNA Synthesis Kit	11754050

Table 6 Compatible PCR Master Mixes

Item	Source
TaqMan [®] Fast Advanced Master Mix	4444556
TaqPath [™] qPCR Master Mix, CG	A16245
TaqMan [®] Gene Expression Master Mix	4369016
TaqMan [®] OpenArray [™] Real-Time PCR Master Mix ^[1]	4462159
TaqMan [®] OpenArray [™] Genotyping Master Mix ^[2]	4404846
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

^[1] Optimized for use with TaqMan[®] OpenArray[™] gene expression panels.

^[2] Optimized for SNP genotyping using TaqMan[®] OpenArray[™] Genotyping Plates.

Table 7 Real-time PCR systems

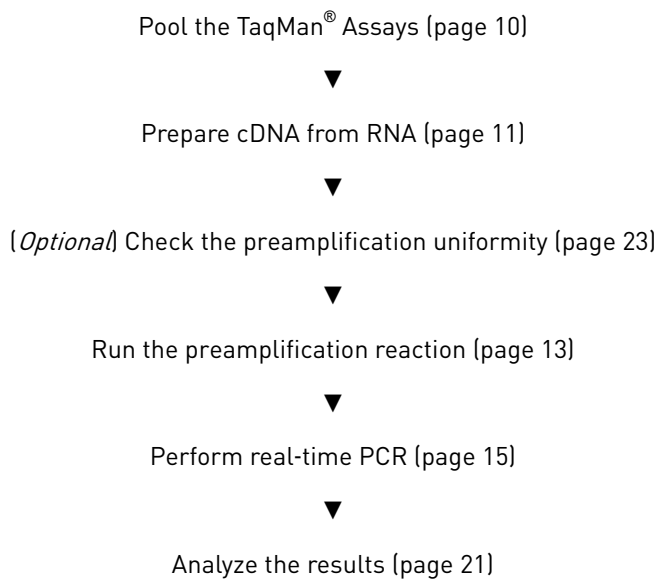
Item	Supported assay formats ^[1]	Source
One of the following:		
QuantStudio [™] 3 Real-Time PCR System	96-well Standard (0.2-mL) Plates or 96-well Fast (0.1-mL) Plates	Contact your local sales office.
7500/7500 Fast Real-Time PCR System		
QuantStudio [™] 5 Real-Time PCR System	96-well Standard (0.2-mL) Plates, 96-well Fast (0.1-mL) Plates, or 384-well plates	
QuantStudio [™] 6 Flex Real-Time PCR System		
QuantStudio [™] 7 Flex Real-Time PCR System	96-well Standard (0.2-mL) Plates, 96-well Fast (0.1-mL) Plates, 384-well plates, or TaqMan [®] Array Cards	
ViiA [™] 7 Real-Time PCR System		
QuantStudio [™] 12K Flex Real-Time PCR System	96-well Standard (0.2-mL) Plates, 96-well Fast (0.1-mL) Plates, 384-well plates, TaqMan [®] Array Cards, or TaqMan [®] OpenArray [™] Plates	
StepOnePlus [™] Real-Time PCR System	96-well Standard (0.2-mL) Plates	
7900HT Fast Real-Time PCR System	TaqMan [®] Array Cards	

^[1] The instrument must be configured with the appropriate block and heated cover.

Table 8 Other required materials

Item	Source
Software	
<i>(Optional)</i> Relative Quantification app	Available on the Thermo Fisher Cloud
<i>(Optional)</i> ExpressionSuite™ Software	Available at thermofisher.com/expressionsuite
Equipment	
Thermal cycler, one of the following (or equivalent): <ul style="list-style-type: none"> • Veriti™ Thermal Cycler • SimpliAmp™ Thermal Cycler • ProFlex™ PCR System 	Contact your local sales office.
Microcentrifuge	MLS
Vortex mixer	MLS
Centrifuge	MLS
Centrifuge (refrigerated)	MLS
Pipettes	MLS
Micropipettes, 100-µL	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	MLS
TE, pH 8.0	AM9849
RNase Inhibitor	N8080119
RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777019
DNase I, Amplification Grade	18068015

Workflow





Pool the TaqMan[®] Assays

Guidelines for pooling TaqMan[®] Assays

- Do not include an assay for 18S RNA in the pool.

IMPORTANT! The 18S gene is a highly expressed gene that negatively affects the uniformity of preamplification.

- TaqMan[®] PreAmp Pools can be used with the following compatible TaqMan[®] Assays:
 - TaqMan[®] Array Plates and Custom TaqMan[®] Array Plates
 - TaqMan[®] Array Cards and Custom TaqMan[®] Array Cards
 - TaqMan[®] OpenArray[™] Plates and Custom TaqMan[®] OpenArray[™] Plates

Note: TaqMan[®] PreAmp Pools do not contain an assay for 18S RNA.

- (Optional) Check the uniformity of preamplification (see Appendix A, “(Optional) Check the preamplification uniformity”).

Pool the TaqMan[®] Assays

If you are using TaqMan[®] PreAmp Pools, proceed to Chapter 3, “Guidelines for preparation of cDNA”.

1. Combine equal volumes of each 20X TaqMan[®] Gene Expression Assay, up to a total of 100 assays, in a microcentrifuge tube.
Example: Combine 50 20X TaqMan[®] Gene Expression Assays at a volume of 10 μ L each, for a total volume of 500 μ L and a concentration of 0.4X per assay.
 2. Dilute the pooled TaqMan[®] Assays using 1X TE Buffer, so that each assay is at a final concentration of 0.2X.
Example: Add 500 μ L of 1X TE Buffer, for a final concentration of 0.2X per assay.
- Note:** We recommend 1X TE Buffer to maintain stability during long-term storage.

3

Guidelines for preparation of cDNA

Guidelines for isolation of high-quality RNA

- See Table 4 on page 6 for recommended RNA isolation kits.
- *(Optional)* Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparation of cDNA templates

- See Table 5 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 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 genomic DNA 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.
- 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.

4

Perform the preamplification reaction

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Guidelines for performing the preamplification reaction

- Follow “Good laboratory practices for PCR and RT-PCR” on page 30.
- Keep all TaqMan® Gene Expression Assays protected from light and stored as indicated until ready for use. Excessive exposure to light may affect the fluorescent probes.
- Before use:
 - Mix the TaqMan® PreAmp Master Mix thoroughly but gently.
 - Thaw the cDNA samples on ice. Resuspend the cDNA samples by vortexing, then centrifuge the tubes briefly.
 - Thaw the pooled TaqMan® Gene Expression Assays on ice. Mix the assays by vortexing, then centrifuge the tubes briefly.
 - If you are using TaqMan® PreAmp Pools, thaw the pools on ice. Mix the pools by vortexing, then centrifuge the tubes briefly.

Before you begin

***(Optional)* Check the preamplification uniformity**

Before performing preamplification with limited biological samples, we recommend checking whether all amplicons are amplified uniformly without bias. See Appendix A, “(Optional) Check the preamplification uniformity”.

Determine the preamplification conditions

Determine whether to perform 10 or 14 preamplification cycles.

- **10 preamplification cycles**
 - Suitable for reactions using lower numbers of pooled assays.
 - Requires a shorter run time.
 - Produces material sufficient for fifty 20- μ L or twenty 50- μ L PCR amplification reactions.
- **14 preamplification cycles**
 - Suitable for reactions using higher numbers of pooled assays.
 - Suitable when samples are extremely limited.
 - Produces material sufficient for two hundred 20- μ L or eighty 50- μ L PCR amplification reactions.

Run the preamplification reaction

1. Prepare each preamplification reaction in an appropriately-sized microcentrifuge tube, depending on the total volume.

Component	Volume per reaction ^[1]	Final concentration
TaqMan [®] PreAmp Master Mix (2X)	25 μ L	1X
Pooled TaqMan [®] Gene Expression Assays, one of the following: <ul style="list-style-type: none"> • Pooled assay mix (0.2X)^[2] • TaqMan[®] PreAmp Pool 	12.5 μ L	0.05X (each assay)
1–250 ng cDNA sample + Nuclease-free Water	12.5 μ L	0.02–5 ng/ μ L
Total	50 μL	—

^[1] The total reaction volume can be between 10 μ L and 50 μ L per sample. Adjust the volume of each component.

^[2] Prepared in "Pool the TaqMan[®] Assays" on page 10.

2. Cap the microcentrifuge tube or seal the plate with optical adhesive film.
3. Mix the reactions by gently inverting the tube or plate, then centrifuge briefly.
4. (Optional) Apply a compression pad to the plate, if required by your real-time PCR system.

5. Set up the thermal cycling conditions for the preamplification reaction.

Step	Temperature	Time	Cycles
Enzyme activation	95°C	10 minutes	Hold
Denature	95°C	15 seconds	10 or 14
Anneal / Extend	60°C	4 minutes	
Enzyme inactivation ^[1]	99°C	10 minutes	Hold
Final	4°C	∞	Hold

^[1] Optional. An enzyme inactivation step can reduce variability in C_t values for high-throughput protocols where there is a delay between preamplification and real-time PCR.

6. Load the plate or tubes into the thermal cycler.

7. Start the run.

Proceed to Chapter 5, “Perform real-time PCR”. Store preamplification products on ice for immediate use, or in aliquots at –25°C to –15°C for up to 7 days.

Perform real-time PCR

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Guidelines for performing real-time PCR

- Follow “Good laboratory practices for PCR and RT-PCR” on page 30.
- Prepare the real-time PCR reactions in an area free of artificial templates and siRNA transfections. High-copy-number templates can easily contaminate the real-time PCR reactions.
- Keep all TaqMan® Gene Expression Assays protected from light and stored as indicated until ready for use. Excessive exposure to light may affect the fluorescent probes.
- Before use:
 - Mix the PCR Master Mix thoroughly but gently.
 - (*Single-tube assays only*) Resuspend the TaqMan® assay mix by vortexing, then centrifuge the tube briefly.
 - Thaw the preamplified cDNA on ice. Resuspend the samples by vortexing, then centrifuge the tubes briefly.
- Prepare the PCR reaction mix before transferring to the reaction plate for thermal cycling and data analysis.

Note: We recommend three replicates of each PCR reaction.
- Configure run documents according to the instructions provided in the real-time PCR instrument resource documents.

Before you begin

Dilute the preamplification products using 1X TE Buffer according to the following table.

Number of preamplification cycles	Dilution factor ^[1]	Final volume of diluted preamplification product
10	1:5 ^[2]	250 µL
14	1:20	1 mL

^[1] Diluted preamplification products can be stored for up to 7 days at -25°C to -15°C.

^[2] Recommended minimum dilution. If needed, a 1:10 dilution can be used.

Prepare the PCR reaction mix

Thaw the preamplified cDNA products on ice. Resuspend the preamplified cDNA products by vortexing, then centrifuge briefly.

1. (*Single-tube assays only*) Thaw the assays on ice, gently vortex to thoroughly mix, then centrifuge briefly to spin down the contents and eliminate air bubbles.
2. Mix the Master Mix thoroughly, but gently.
3. Prepare PCR Reaction Mix in an appropriately-sized microcentrifuge tube according to one of the following tables.

Note: Three replicates of each PCR reaction are recommended.

Table 9 Single-tube assays (96-well Standard (0.2-mL) Plate)

Component	Volume	
	1 well	3 wells ^[1]
TaqMan [®] Gene Expression Assay (20X)	1.0 µL	3.75 µL
Preamplified cDNA products (diluted 1:5 or 1:20)	5.0 µL	18.75 µL
Master Mix (2X) ^[2]	10.0 µL	37.5 µL
Nuclease-free Water	4.0 µL	15.0 µL
Total PCR Reaction Mix volume	20 µL	75 µL

^[1] Includes 25% overage.

^[2] Table 6 on page 7 for compatible Master Mixes.

Table 10 Single-tube assays (384-well plate or 96-well Fast (0.1-mL) Plate)

Component	Volume	
	1 well	3 wells ^[1]
TaqMan [®] Gene Expression Assay (20X)	0.5 µL	1.88 µL
Preamplified cDNA products (diluted 1:5 or 1:20)	2.5 µL	9.38 µL
Master Mix (2X) ^[2]	5.0 µL	18.75 µL
Nuclease-free Water	2 µL	7.5 µL
Total PCR Reaction Mix volume	10 µL	37.5 µL

^[1] Includes 25% overage.

^[2] Table 6 on page 7 for compatible Master Mixes.

Table 11 96-well Standard (0.2-mL) TaqMan[®] Array Plates, TaqMan[®] Gene Expression Assays

Component	Volume	
	1 well	3 wells ^[1]
Preamplified cDNA products (diluted 1:5 or 1:20)	5.0 µL	18.75 µL
Master Mix (2X) ^[2]	10.0 µL	37.5 µL
Nuclease-free Water	5.0 µL	18.75 µL
Total PCR Reaction Mix volume	20 µL	75 µL

^[1] Includes 25% overage.

^[2] Table 6 on page 7 for compatible Master Mixes.

Table 12 96-well Fast (0.1-mL) TaqMan[®] Array Plates or 384-well plates, TaqMan[®] Gene Expression Assays

Component	Volume	
	1 well	3 wells ^[1]
Preamplified cDNA products (diluted 1:5 or 1:20)	2.5 µL	9.38 µL
Master Mix (2X) ^[2]	5.0 µL	18.74 µL
Nuclease-free Water	2.5 µL	9.38 µL
Total PCR Reaction Mix volume	10 µL	37.5 µL

^[1] Includes 25% overage.

^[2] Table 6 on page 7 for compatible Master Mixes.

Table 13 TaqMan® Array Cards, TaqMan® Gene Expression Assays

Component	Volume per fill reservoir ^[1]
Preamplified cDNA products (diluted 1:5 or 1:20)	55 µL
Master Mix (2X) ^[2]	55 µL
Total PCR Reaction Mix volume	110 µL

^[1] Includes 10% overage.

^[2] Table 6 on page 7 for compatible Master Mixes.

Table 14 OpenArray™ format, TaqMan® Gene Expression Assays

Component	Volume (per subarray) ^[1]	Volume (1 sample per 2 subarrays) ^[2,3]	Volume (1 sample per 3 subarrays) ^[2, 4]
Preamp. cDNA products (diluted 1:5 or 1:20)	2.5 µL	6.25	9.38 µL
TaqMan® OpenArray™ Real-Time PCR Master Mix (2X)	2.5 µL	6.25	9.38 µL
Total PCR Reaction Mix volume	5 µL	12.5 µL	18.75 µL

^[1] Full volume is added to a single well of an OpenArray™ 384-well Sample Plate.

^[2] Includes 25% overage.

^[3] 5 µL is added to each of 2 wells of an OpenArray™ 384-well Sample Plate.

^[4] 5 µL is added to each of 3 wells of an OpenArray™ 384-well Sample Plate.

Table 15 OpenArray™ format, TaqMan® SNP Genotyping Assays

Component	Volume (per subarray) [1]	Volume (1 sample per 2 subarrays) [2,3]	Volume (1 sample per 3 subarrays) [2, 4]
Preamp. cDNA products (diluted 1:5 or 1:20)	2.5 µL	6.25	9.38 µL
TaqMan® OpenArray™ Genotyping Master Mix (2X)	2.5 µL	6.25	9.38 µL
Total PCR Reaction Mix volume	5 µL	12.5 µL	18.75 µL

[1] Full volume is added to a single well of an OpenArray™ 384-well Sample Plate.

[2] Includes 25% overage.

[3] 5 µL is added to each of 2 wells of an OpenArray™ 384-well Sample Plate.

[4] 5 µL is added to each of 3 wells of an OpenArray™ 384-well Sample Plate.

4. Pipette gently to mix the contents thoroughly, then cap the tubes.
5. Centrifuge briefly to collect the contents at the bottom of the tubes.

Prepare the PCR reaction plate

See the appropriate user guide if you are using TaqMan® Array Plates, TaqMan® Array Cards, or TaqMan® OpenArray™ Plates (see “Related documentation” on page 34).

1. Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.
2. Seal the plate with optical adhesive film or with optical flat caps.
3. Centrifuge briefly to bring the PCR Reaction Mix to the bottom of the well and eliminate air bubbles.
4. Apply a compression pad to the plate, if required by your real-time PCR system.

Set-up and run the real-time PCR

IMPORTANT! The thermal protocols listed in this section are for plates (with single-tube assays or dried-down assays) and TaqMan® Fast Advanced Master Mix or TaqPath™ qPCR Master Mix, CG. See “Thermal protocols” on page 26 for the thermal protocols for other assay formats and Master Mixes.

If you are using TaqMan® OpenArray™ Plates, follow the appropriate user guide (see “Related documentation” on page 34).

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 or card.

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 16 TaqMan® Fast Advanced Master Mix or TaqPath™ qPCR Master Mix, CG (QuantStudio™ system, ViiA™ 7, StepOnePlus™ Real-Time PCR System, or 7900HT Fast Real-Time PCR Instrument)

Step	Temperature	Time (fast cycling mode)	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] For optimal UNG activity.

^[2] Enzyme activation time can be up to 2 minutes (TaqMan® Fast Advanced Master Mix) or up to 10 minutes (TaqPath™ qPCR Master Mix, CG). A longer enzyme activation time can help to denature double-stranded genomic DNA templates. The enzyme activation time should not affect results if a preamplified cDNA template is used.

Table 17 TaqMan® Fast Advanced Master Mix or TaqPath™ qPCR Master Mix, CG (7500/7500 Fast Real-Time PCR System)

Step	Temperature	Time (fast cycling mode)	Cycles
UNG incubation ^[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds ^[2]	1

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

^[1] For optimal UNG activity.

^[2] Enzyme activation time can be up to 2 minutes (TaqMan® Fast Advanced Master Mix) or up to 10 minutes (TaqPath™ qPCR Master Mix, CG). A longer enzyme activation time can help to denature double-stranded genomic DNA templates. The enzyme activation time should not affect results if a preamplified cDNA template is used.

3. Set the reaction volume appropriate for the reaction plate.
 - **96-well Standard (0.2-mL) Plate**—20 µL
 - **96-well Fast (0.1-mL) Plate**—10 µL
 - **384-well plate**—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 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 app	thermofisher.com/cloud
ExpressionSuite™ Software ^[1]	thermofisher.com/expressionsuite

^[1] Can automatically define the baseline.

For more information about real-time PCR, see page 34 or go to thermofisher.com/qpcr/education.

Data can be analyzed using the relative threshold algorithm (C_{rt}). The C_{rt} algorithm is recommended for TaqMan® Array Cards. It can correct a variable baseline, which can be due to dried-down assays on the card being reconstituted at different rates. The C_{rt} can also be used for single-tube assays and TaqMan® Array Plates.

Use the relative threshold algorithm in your software. If your software does not have the relative threshold algorithm, you can use the Relative Quantification app that is available on the Thermo Fisher Cloud.

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



(Optional) Check the preamplification uniformity

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We recommend checking whether all amplicons are amplified uniformly without bias before you perform preamplification with your limited biological samples. Checking preamplification uniformity involves performing a relative quantitation experiment with your selected TaqMan® Gene Expression Assays to compare amplification of cDNA to amplification of preamplified cDNA.

We recommend checking preamplification uniformity using cDNA that is non-limiting, such as a control sample, and that was prepared using a recommended cDNA synthesis kit (see Table 5 on page 6).

Prepare preamplified cDNA

1. Pool the TaqMan® Gene Expression Assays as described on page 10.
Note: Make sure to include an endogenous uniformity reference gene in the pool. For human gene expression assays, we recommend using CDKN1B (Assay ID HS00153277_m1) because of its consistent gene expression profile.
2. Using a non-limited cDNA sample, prepare preamplified cDNA according to Chapter 4, “Perform the preamplification reaction”.

Prepare the uniformity reaction plate

For each TaqMan® Gene Expression Assay in the pool, set up two sets of separate reactions using the procedures in “Prepare the PCR reaction mix” on page 16 and “Prepare the PCR reaction plate” on page 19:

- **cDNA:** Use 0.3 ng/μL of the non-limited cDNA sample that is not preamplified.
- **Preamplified cDNA:** Use diluted preamplified non-limited cDNA.



Run the uniformity plate

Perform a relative quantitation run on a real-time PCR system according to “Set-up and run the real-time PCR” on page 20.

Analyze the uniformity results

Use a relative quantitation study to analyze your results and to determine $\Delta\Delta C_t$ values between the cDNA plate (cDNA that is not preamplified) and the preamplified cDNA plate. See “Related documentation” on page 34 for the appropriate relative quantitation document.

Note: C_q is used to refer to cycles generically. C_t and C_{rt} are used to differentiate between the traditional baseline threshold method and the relative threshold method, respectively.

1. Calculate the average C_q values for each assay.

Note: Set the cDNA plate as the calibrator under analysis settings.

2. Calculate ΔC_q for cDNA by subtracting the average C_q value of the uniformity reference gene from the average C_q value of each gene.

$$\Delta C_q (\text{cDNA}) = \text{avg } C_q (\text{Target X}) - \text{avg } C_q (\text{Uniformity ref gene})$$

The purpose is to normalize each individual target to the desired uniformity reference gene when using cDNA that is not preamplified.

3. Calculate ΔC_q for multiplex preamplification by subtracting the average C_q value of the uniformity reference gene from the average C_q value of each gene.

$$\Delta C_q (\text{Preamp}) = \text{avg } C_q (\text{Target X}) - \text{avg } C_q (\text{Uniformity ref gene})$$

The purpose is to normalize each individual target to the desired uniformity reference gene when using preamplified cDNA.

4. Calculate $\Delta\Delta C_q$ between cDNA and preamplified cDNA by subtracting the ΔC_q value for cDNA (step 2) from the ΔC_q for multiplex preamplification (step 3).

$$\Delta\Delta C_q = \Delta C_q (\text{Preamp}) - \Delta C_q (\text{cDNA})$$

A $\Delta\Delta C_q$ value close to zero indicates preamplification uniformity. Typically, 90% of targets produce $\Delta\Delta C_q$ values within ± 1.5 .

Example: Preamplification uniformity results

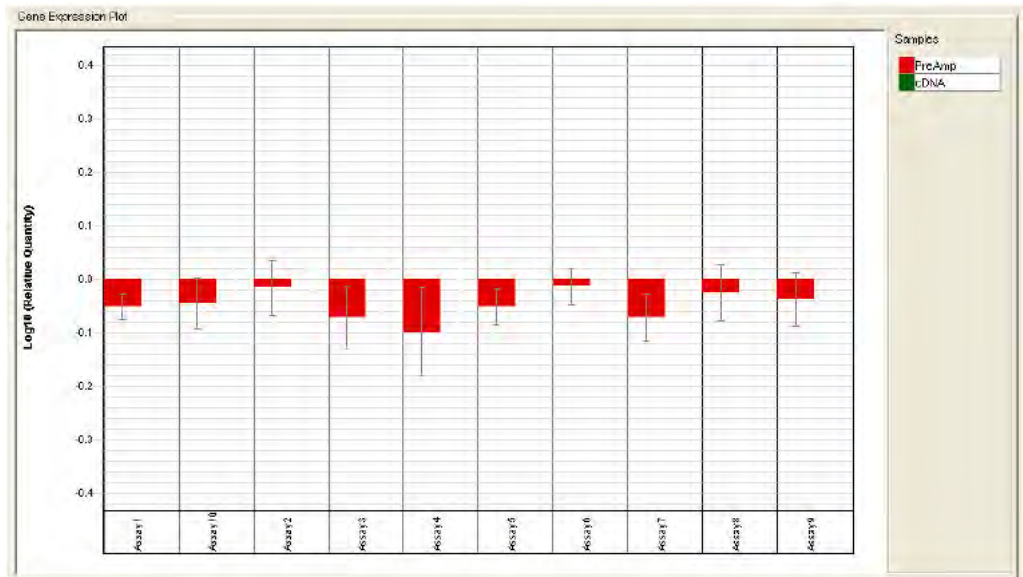


Figure 1 Log₁₀ of fold difference between preamplified cDNA and non-preamplified cDNA for 10 assays. All assays show preamplification uniformity [$\Delta\Delta C_t$ within ± 1.5 , or Log₁₀ of $\Delta\Delta C_t$ within ± 0.452].



Supplemental information

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” on page 20 are optimized for plates (with single-tube assays or dried-down assays) and TaqMan® Fast Advanced Master Mix or TaqPath™ qPCR Master Mix, CG.

The following tables provide thermal protocols for other Master Mixes that are compatible with the following assay formats:

- Single-tube assays and TaqMan® Array Plates
- TaqMan® Array Cards

IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction.

Single-tube assays and TaqMan® Array Plates

Table 18 TaqMan® Universal Master Mix II, with UNG or TaqMan® Gene Expression Master Mix (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 19 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 20 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (QuantStudio™ system, ViiA™ 7, StepOnePlus™ Real-Time PCR System, or 7900HT Fast Real-Time PCR Instrument)

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 21 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (7500/7500 Fast Real-Time PCR 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	

TaqMan® Array Cards

Table 22 TaqMan® Fast Advanced Master Mix (ViiA™ 7 and compatible QuantStudio™ systems)

Step	Temp.	Time (fast cycling mode)	Ramp rate	Cycles
UNG incubation ^[1]	50°C	2 minutes	1.75°C/second	1
Enzyme activation	92°C	10 minutes ^[2]	1.75°C/second	1
Denature	95°C	1 second	1.75°C/second	40
Anneal / Extend	60°C	20 seconds	1.83°C/second	

^[1] For optimal UNG activity.

^[2] To completely dissolve primers and probes on the card.

Table 23 TaqMan® Fast Advanced Master Mix (7900HT Fast Real-Time PCR Instrument)

Step	Temperature	Time (fast cycling mode)	Cycles
UNG incubation ^[1]	50°C	2 minutes	1
Enzyme activation	92°C	10 minutes ^[2]	1
Denature	97°C	1 second	40
Anneal / Extend	62°C	20 seconds	

^[1] For optimal UNG activity.^[2] To completely dissolve primers and probes on the card.**Table 24** TaqMan® Gene Expression Master Mix and 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 ^[2]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

^[1] For optimal UNG activity.^[2] To completely dissolve primers and probes on the card.**Table 25** TaqMan® Universal Master Mix II, no UNG (any compatible instrument)

Step	Temperature	Time (standard cycling mode)	Cycles
Enzyme activation	95°C	10 minutes ^[1]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

^[1] To completely dissolve primers and probes on the card.

Table 26 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (ViiA™ 7 and compatible QuantStudio™ systems)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	92°C	10 minutes ^[1]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

^[1] To completely dissolve primers and probes on the card.

Table 27 TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG (7900HT Fast Real-Time PCR Instrument)

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	92°C	10 minutes ^[1]	1
Denature	97°C	1 second	40
Anneal / Extend	60°C	20 seconds	

^[1] To completely dissolve primers and probes on the card.

Best practices for PCR and RT-PCR experiments

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- 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.



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, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
-



Chemical safety



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

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



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:
www.cdc.gov/biosafety/publications/bmb15/BMBL.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.
<i>Introduction to Gene Expression Getting Started Guide</i>	4454239
<i>TaqMan[®] PreAmp Master Mix Quick Reference</i>	4384556
<i>High-Capacity cDNA Reverse Transcription Kit Protocol</i>	4375575
<i>SuperScript[™] VIL0[™] cDNA Synthesis Kit Product Information Sheet</i>	MAN0000749
<i>TaqMan[®] Gene Expression Assays User Guide—single-tube assays</i>	4333458
<i>TaqMan[®] Gene Expression Assays Quick Reference—single-tube assays</i>	4401212
<i>TaqMan[®] Gene Expression Assays User Guide—TaqMan[®] Array Plates</i>	4391016
<i>TaqMan[®] Gene Expression Assays Quick Reference—96-well Standard (0.2-mL) Plates</i>	4391139
<i>TaqMan[®] Gene Expression Assays Quick Reference—96-well Fast (0.1-mL) Plates</i>	4427562
<i>TaqMan[®] Gene Expression Assays User Guide—TaqMan[®] Array Cards</i>	4400263
<i>TaqMan[®] Gene Expression Assays Quick Reference—TaqMan[®] Array Cards</i>	4371129
<i>TaqMan[®] SNP Genotyping Assays User Guide</i>	MAN0009593
<i>QuantStudio[™] 12K Flex Real-Time PCR System: OpenArray[™] Experiments User Guide</i>	4470935
<i>Understanding Your Shipment</i>	MAN0017153
QuantStudio[™] 3 or 5 Real-Time PCR System	
<i>QuantStudio[™] 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>QuantStudio[™] Design and Analysis desktop Software User Guide</i>	MAN0010408
QuantStudio[™] 6 / QuantStudio[™] 7 Flex Real-Time PCR System	
<i>QuantStudio[™] 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i>	4489821
<i>QuantStudio[™] 6 and 7 Flex Real-Time PCR System Software Getting Started Guide</i>	4489822
QuantStudio[™] 12K Flex Real-Time PCR System	
<i>QuantStudio[™] 12K Flex Real-Time PCR System Maintenance and Administration Guide</i>	4470689
<i>QuantStudio[™] 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide</i>	4470050

Document	Pub. No.
StepOnePlus™ Real-Time PCR System	
<i>StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide</i>	4376782
<i>Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide</i>	4376785
ViiA™ 7 Real-Time PCR System	
<i>Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security</i>	4442661
<i>Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide</i>	4441434
7500/7500 Fast Real-Time PCR System	
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</i>	4347828
<i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments</i>	4387783
Real-time PCR chemistry guide	
<i>Applied Biosystems™ Real-Time PCR Systems Chemistry Guide</i>	4348358
Resources for data analysis	
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve</i>	4347825
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Quantitation using Comparative C_t</i>	4347824
<i>Applied Biosystems™ 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide</i>	4364016
<i>Applied Biosystems™ Relative Quantitation Analysis Module User Guide</i>	MAN0014820
<i>Applied Biosystems™ Genotyping Analysis Module User Guide</i>	MAN0014821

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 - Safety Data Sheets (SDSs; also known as MSDSs)

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

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