TaqMan® PreAmp Master Mix

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® PreAmp Master Mix User Guide* (Pub. No. 4384557). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of TaqMan® PreAmp Master Mix. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® PreAmp Master Mix User Guide* (Pub. No. 4384557).

Pool the TaqMan® Assays

Do not include an assay for 18S RNA in the pool. The 18S gene is a highly expressed gene that negatively affects the uniformity of preamplification.

If you are using TaqMan® PreAmp Pools, proceed to "Prepare cDNA from RNA".

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 µL each, for a total volume of 500 µL 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 µL 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.

Prepare cDNA from RNA

We recommend the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (Cat. No. 4368813) or the SuperScript™ VILO™ cDNA Synthesis Kit (Cat. No. 11754050).

Run the preamplification reaction

Before performing preamplification, determine whether to perform 10 or 14 preamplification cycles (see the *TaqMan® PreAmp Master Mix User Guide*, Pub. No. 4384557).

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 µL	1X
Pooled assay mix (0.2X, each assay) or TaqMan® PreAmp Pool	12.5 µL	0.05X (each assay)
1–250 ng cDNA sample + Nuclease-free Water	12.5 µL	0.02–5 ng/µL
Total	**50 µL**	**—**

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

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 "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

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 1 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)	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.

Table 2 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)	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.

Table 3 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)	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.

Table 4 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)	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.

Table 5 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)	55 µL
Total PCR Reaction Mix volume	**110 µL**

[1] Includes 10% overage.

Table 6 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 7 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

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 4 for the thermal protocols for other assay formats and Master Mixes.

If you are using TaqMan® OpenArray™ Plates, follow the appropriate user guide.

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 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 9 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
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.

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.

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 3 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 10 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 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 (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 13 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 14 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 15 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 16 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 17 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 18 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 19 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.

Limited product warranty

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

Revision history: Pub. No. 4384556

Revision	Date	Description
C	11 July 2018	• Updated enzyme inactivation step in preamplification to be optional. • Updated 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.
B	1 February 2018	• Added an enzyme inactivation step to the preamplification conditions. • Rebranded document. Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A	March 2007	New document.