TaqMan® PrepAmp Pools

For safety and biohazard guidelines, refer to the "Safety Information" in the *TaqMan® PrepAmp Pools Protocol* (PN 4397427). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Workflow

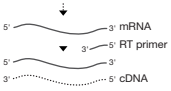
Prepare your samples

Isolate the total RNA

For recommendations, refer to the Ambion RNA Isolation Decision Tree: www.ambion.com/techlib/trees/RNA/index.html

Evaluate the total RNA

Perform reverse transcription



High Capacity RNA-to-cDNA Kit
or
High Capacity cDNA Reverse Transcription Kit

(Optional) Evaluate the cDNA

Perform the preamplification reactions

Prepare the preamplification reaction

Perform thermal cycling

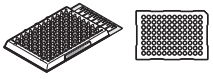
A thermal cycler, for example, Veriti Thermal Cycler

Perform the amplification reactions

Prepare the reaction mix

A TaqMan® master mix

Prepare the TaqMan® Array or TaqMan® Express Plate

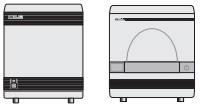


TaqMan® Array or
TaqMan® Express Plate

Set up the SDS plate document

System software and information CD

Perform the run (qPCR)



Real-Time PCR System

Analyze the experiment

Perform a Comparative C_T ($\Delta\Delta C_T$) Experiment

1 Prepare Your Samples

a. Isolate total RNA, then evaluate the total RNA.

b. Perform reverse transcription to convert total RNA to cDNA. Applied Biosystems recommends the:

- High Capacity RNA-to-cDNA Kit
- High Capacity RNA-to-cDNA Master Mix
- High Capacity cDNA Reverse Transcription Kit

c. (Optional) Evaluate the cDNA.

2 Perform the Preamplification Reactions

Prepare the Preamplification Reactions

a. Determine the total number of preamplification reactions to be run.

b. Per the table below, calculate the total volume required for each reaction component: volume for 1 reaction × the total number of reactions. Include 12.5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Note: If you need to run replicates of the same preamplification products, scale up this reaction volume accordingly. Each preamplification reaction will be used for 2 fill reservoirs of 96 stem cell pluripotency assays or 8 fill reservoirs of 384 GPCR assays.

Preamplification Reaction Component	Minimum Volume (µL) for 1 Reaction
TaqMan® PreAmp Master Mix	15.0
TaqMan® PreAmp Pools	7.5
cDNA (0.2 to 100 ng) + nuclease-free water	7.5
Total Volume	**30.0**

c. On ice, thaw the TaqMan PreAmp Master Mix and TaqMan PreAmp Pools. Gently invert the tubes to mix, spin briefly, then return the tubes to ice.

d. On ice, in a 0.2-mL reaction tube, tube strip, or 96-well optical reaction plate, combine the required volumes of each reaction component.

e. Cap the reaction tube or seal the reaction plate.

Note: If you are running a reaction plate on a 7900HT Fast System, seal the plate with MicroAmp™ Clear Adhesive Film, then place a MicroAmp™ Optical Film Compression Pad on top of the reaction plate.

f. Invert the reaction tube or plate several times to mix, then briefly centrifuge.

2 Perform the Preamplification Reactions

Perform Thermal Cycling

a. Load the reaction tube or plate into the instrument.

b. Set up the run method using the following conditions:

- Reaction volume (µL): **30**
- Ramp speed or mode: **Standard**
- Thermal profile:

Hold	Cycle (14 cycles)		Hold
	Denature	Anneal/Extend	
95 °C	95 °C	60 °C	4 °C
10 min	15 sec	4 min	∞

c. After the run is complete, unload the reaction tube or plate. Proceed directly to "Perform the Amplification Reactions" on page 3, or store the preamplified product at –20 °C for up to 2 weeks.

IMPORTANT! Applied Biosystems recommends running the TaqMan Arrays within 24 hours after loading the preamplification product. See "Perform the Amplification Reactions" below.

3 Perform the Amplification Reactions

Prepare the Reaction Mix

a. If the preamplified cDNA is frozen, thaw it on ice. To resuspend the preamplified cDNA, invert the tube several times, then briefly centrifuge the tube.

b. Mix the TaqMan master mix thoroughly by swirling the bottle.

c. In a microcentrifuge tube, combine the required volumes for all reaction components, per the table below.

Amplification Reaction Component	Volume (µL)	
	Format 96 Arrays (for 2 fill reservoirs, 100 µL/reservoir)	Format 384 Arrays (for 8 fill reservoirs, 100 µL/reservoir)
Preamplified cDNA	27.5	27.5
Nuclease-free water	82.5	412.5
TaqMan® Gene Expression Master Mix *or* **TaqMan® Universal PCR Master Mix**	110.0	440.0
Total Volume	220.0	880.0

IMPORTANT! The recommended minimum dilution for any Format 96 Array is 1:8. The recommended minimum dilution for any Format 384 Array is 1:32. Adding more preamplified cDNA increases the fluorescence baseline and may result in problems with analysis.

d. Cap the tube, then gently vortex to thoroughly mix the solution.

e. Briefly centrifuge the tube to spin down the contents and eliminate air bubbles.

3 Perform the Amplification Reactions

Prepare the Array

a. Allow the TaqMan Array to reach room temperature (~15 minutes), then carefully remove it from its packaging.

b. Add 100 µL of the reaction mix to each fill reservoir of the TaqMan Array.

c. Centrifuge, then seal and trim the array.

Set Up the SDS Plate Document and Perform the Run (qPCR)

a. In the SDS software (v2.1 or later), set up the SDS plate document.

b. Load the TaqMan Array into a 7900HT Fast instrument that has a TaqMan® Array Thermal Cycling Block installed.

c. Start the run using the default thermal-cycling conditions.

4 Analyze the Experiment

Note: See Appendix B of the *TaqMan® PrepAmp Pools Protocol*, "Determination of Preamplification Uniformity and Sensitivity."

a. Transfer the SDS plate document file (*.sds) into an RQ Study, then analyze the study. For optimal results, Applied Biosystems recommends the following:

- For Applied Biosystems TaqMan master mixes, analyze the study with Automatic Baseline and Manual C_T set to **0.2**.
- View the amplification plot, then review the baseline and threshold settings. If needed, adjust the baseline and threshold settings.

  IMPORTANT! The same threshold setting must be used for an assay across all samples or arrays within a study.

  Note: You can use either TaqMan Universal PCR Master Mix or TaqMan Gene Expression Master Mix in your experiment. However, Applied Biosystems strongly recommends that you use only one type of master mix per study.

b. In the well table or results table, review the C_T values for each well and for each replicate group. If needed, omit outliers.

c. Review the gene expression plot (for SDS Software v2.3, view the amplification plots in the Plate, Detector, or Sample view).

Available TaqMan® PreAmp Pools and TaqMan® Arrays

The TaqMan Array must contain the TaqMan Gene Expression Assays that target the same genes as your TaqMan PreAmp Pool, with the exception of the 18S gene. Applied Biosystems does not include the 18S gene in the PreAmp Pool because it is a high-expressing gene that negatively affects the uniformity of preamplification.

Product		Part Number
TaqMan® PreAmp Pools	**Human** GPCR	4385234
	Rat GPCR	4385109
	Human stem cell pluripotency	4385110
	Mouse stem cell pluripotency	4385111
TaqMan® Arrays	TaqMan® **Human** GPCR Array	4367785
	TaqMan® **Rat** GPCR Array	4378709
	TaqMan® **Human** Stem Cell Pluripotency Array	4385344
	TaqMan® **Mouse** Stem Cell Pluripotency Array	4385363

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