TaqMan® Small RNA Assays

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

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® Small RNA Assays Protocol* (Part no. 4364031). For all chemicals, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Perform reverse transcription (RT)

1 Prepare the total RNA

a. Isolate the total RNA.

Use a method that preserves small RNAs. To prevent the loss of the longer control transcripts (snoRNAs), we recommend that you do *not* perform size fractionation.

b. Quantify the amount of total RNA in your sample.

2 Prepare the RT master mix

a. Allow the TaqMan® MicroRNA Reverse Transcription Kit components to thaw on ice.

b. In a polypropylene tube, prepare the RT master mix on ice by scaling the volumes listed below to the desired number of RT reactions. We recommend adding 10–20% overage to account for pipetting losses.

Component	Master mix volume per 15-µL reaction†
100mM dNTPs (with dTTP)	0.15 µL
MultiScribe™ Reverse Transcriptase, 50 U/µL	1.00 µL
10× Reverse Transcription Buffer	1.50 µL
RNase Inhibitor, 20 U/µL	0.19 µL
Nuclease-free water	4.16 µL
Total volume	**7.00 µL**

† Each 15-µL RT reaction consists of 7 µL master mix, 3 µL of 5X RT primer, and 5 µL RNA sample.

c. Mix gently. Centrifuge to bring solution to the bottom of the tube.

d. Place the RT master mix on ice until you prepare the RNA reaction.

3 Prepare the RT reaction

a. Thaw the 5× RT primer and RNA template on ice. Before use, vortex the RT primer tubes to mix, then centrifuge briefly.

b. If you are performing quantitation of:

- Ambion *Silencer*® Select siRNAs, go to step c.
- All other templates, go to step d.

3 Prepare the RT reaction *(continued)*

c. If you are performing Ambion *Silencer*® Select siRNA quantitation, denature and prepare the double-stranded template:

1. For each 15-µL RT reaction, combine 3 µL of 5X RT primer and 5 µL of double-stranded template in a 0.2-mL reaction tube or a well of a 96-well reaction plate.
2. Incubate the tube or plate at 85°C for 5 minutes, then at 60°C for 5 minutes.
3. Place the denatured template on ice.
4. For each 15-µL RT reaction, combine RT master mix with denatured RNA and RT primer in the ratio of:

   7 µL RT master mix : 8 µL denatured RNA and RT primer (1–10 ng of RNA per reaction)
5. Go to step e.

d. If you are preparing single-stranded RNA, prepare the total RNA template:

1. For each 15 µL RT reaction, combine RT master mix with 1–10 ng total RNA in the ratio of:

   7 µL RT master mix : 5 µL total RNA
2. Mix gently, then centrifuge briefly to bring the solution to the bottom of the tube.
3. Transfer 12.0 µL of the RT master mix-total RNA mixture to a 0.2-mL reaction tube or a well of a 96-well reaction plate.
4. Add 3 µL of 5-µl RT primer from each assay set to the corresponding RT reaction tube or well.
5. Go to step e.

e. Seal the reaction tube or reaction plate, then mix gently. Centrifuge briefly.

f. Incubate the reactions on ice for 5 minutes, then keep on ice until you load the thermal cycler.

4 Perform reverse transcription

Load the reaction tubes or plate into a thermal cycler, then perform a run using the following conditions:

- Mode: Standard
- Reaction volume: 15 µL
- Thermal cycling conditions:

Step	Time	Temperature
Hold	30 minutes	16°C
Hold	30 minutes	42°C
Hold	5 minutes	85°C
Hold	∞	4°C

Quantitative PCR (qPCR) amplification

1 Prepare the qPCR reactions

a. Place the components on ice. Gently invert the tubes to mix, then return them to the ice.

b. Calculate the volumes needed based on the number of reactions and a reaction volume of 20 µL.

Note: We recommend performing three replicates of each reaction. Include excess volume in your calculations to account for loss that occurs during reagent transfers.

Component	Volume (µL)	
	20-µL reaction	3 replicates + 20% excess
TaqMan® Universal PCR Master Mix II, no UNG†	10.00 µL	36.00 µL
Nuclease-free water	7.67 µL	27.61 µL
TaqMan® Small RNA Assay (20X)	1.00 µL	3.60 µL
Product from RT reaction	1.33‡ µL	4.80 µL
Total volume	**20.00 µL**	**72.01 µL**

† TaqMan Universal PCR Master Mix II with UNG is compatible with TaqMan® Small RNA Assays.
‡ Maximum volume of RT product that can be added to each reaction.

c. Combine reaction components in a microcentrifuge tube.

d. Mix gently by inversion, then centrifuge the tube or reaction plate briefly.

2 Prepare the qPCR reaction plate

a. Transfer 20 µL of the PCR reaction into wells of a reaction plate.

b. Seal the plate with optical adhesive film or optical caps, then briefly centrifuge the reaction plate.

3 Run the PCR reaction plate

a. Create an experiment or plate document using the following parameters:

- Mode: Standard
- Reaction volume: 20 µL
- Thermal cycling conditions:

Step	Optional AmpErase® UNG activity†	Enzyme Activation	PCR	
	HOLD	HOLD	CYCLE (40 cycles)	
			Denature	Anneal/extend
Temperature	50°C	95°C	95°C	60°C
Time	2 minutes	10 minutes	15 seconds	60 seconds

† Not needed when UNG is not in the reaction.

b. Load the reaction plate into the real-time PCR instrument.

c. Start the run.

4 Analyze the experiment

Refer to the getting started guides for your real-time PCR system to analyze the experiment. The general process for analyzing the data from gene expression assays involves the following procedures:

a. View the amplification plots.

b. Set the baseline and threshold values.

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NOTICE TO PURCHASER: PLEASE REFER TO THE TAQMAN® SMALL RNA ASSAYS SUBMISSION GUIDELINES PROTOCOL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION

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