TaqMan® Pri-miRNA Assays

For detailed instructions on using TaqMan® Pri-miRNA Assays, refer to the *TaqMan® Pri-miRNA Assays Protocol* (PN 4427719). For safety and biohazard guidelines, refer to the "Safety" section in the protocol. For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1 Prepare the cDNA sample

a. Isolate total RNA. Applied Biosystems recommends using an Ambion® RNA isolation kit.

IMPORTANT! If your RNA purification method does not include DNase treatment, treat the purified RNA with the Ambion TURBO DNA-*free*™ Kit (PN AM1907).

b. Perform reverse transcription (RT). Applied Biosystems recommends using the High Capacity RNA-to-cDNA Kit (PN 4387406) or the High Capacity cDNA Reverse Transcription Kit (PN 4368813, 4374966).

Use the same RT procedure for all samples in an experimental study.

c. Store the cDNA samples at –15 to –25 °C, if you do not proceed immediately to PCR.

2 Prepare the PCR reaction mix

Use the same amount of cDNA for all samples (1 to 100 ng per 20-µL reaction).

a. For each sample (to be run in quadruplicate), pipette the following into a nuclease-free 1.5-mL microcentrifuge tube:

PCR reaction mix component	Volume per 20-µL reaction (µL)	
	Single reaction	Four replicates‡
20× TaqMan® Pri-miRNA Assays	1.0	5.0
2× TaqMan® Gene Expression Master Mix§	10.0	50.0
cDNA template (1 to 100 ng)#	4.0	20.0
RNase-free water	5.0	25.0

‡ Replicate volumes include 20% excess for volume loss from pipetting.
§ (Optional) Use **TaqMan® Fast Universal Master Mix (2×), No AmpErase® UNG** or **TaqMan® Universal Master Mix**. If you add AmpErase® UNG (uracil-N-glycosylase), the final concentration must be 0.01 U/µL.
# Applied Biosystems recommends that no more than 20% of the PCR be composed of the reverse transcription reaction.

b. Cap the tube and invert it several times to mix the reaction components.

c. Centrifuge the tube briefly.

3 Load the plate

a. Transfer 20 µL of PCR reaction mix into each well of a 48-, 96-, or 384-well reaction plate.

b. Seal the plate with the appropriate cover.

c. Centrifuge the plate briefly.

IMPORTANT! If you use TaqMan® Fast Universal PCR Master Mix (2×), run the reaction plate within 2 hours of completing the reaction setup. Otherwise, refrigerate or freeze the plate until you can load it into the instrument.

d. Load the plate into the instrument.

4 Run the plate

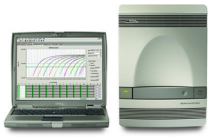
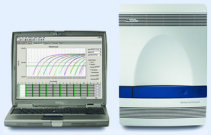
a. Create an experiment/plate document for the run using the parameters shown in Table 1.

b. Run the plate.

5 Analyze the results

Refer to the user guide for your real-time PCR instrument.

Table 1 Plate document/experiment parameters for TaqMan® Pri-miRNA Assays

System	Run	Reaction plate	Plate document/ experiment parameters	Thermal cycling conditions		
				Stage	Temp (°C)	Time (mm:ss)
Applied Biosystems 7300/7500 Real-Time PCR System 	Standard	96-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard‡	Hold§	50	2:00
				Hold	95	10:00
				Cycle (40 Cycles)	95	0:15
					60	1:00
Applied Biosystems 7500 Fast Real-Time PCR System 	Standard	96-well Fast	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Hold§	50	2:00
				Hold	95	10:00
				Cycle (40 Cycles)	95	0:15
					60	1:00
	Fast	96-well Fast	• Rxn. Volume: 20 µL • Ramp Rate: Fast	Hold§	50	2:00
				Hold	95	0:20
				Cycle (40 Cycles)	95	0:03
					60	0:30
Applied Biosystems 7900HT Real-Time PCR System 	Standard	96-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Hold§	50	2:00
				Hold	95	10:00
		384-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Cycle (40 Cycles)	95	0:15
					60	1:00
	Fast	96-well Fast	• Rxn. Volume: 20 µL • Ramp Rate: Fast	Hold§	50	2:00
				Hold	95	0:20
		384-well standard	• Rxn. Volume: 20 µL • Ramp Rate: Standard	Cycle (40 Cycles)	95	0:01
					60	0:20
Applied Biosystems StepOne™/ StepOnePlus™ Real-Time PCR System 	Standard	48/96-well Fast	• Rxn. Volume: 20 µL • Ramp Speed: Standard	Hold§	50	2:00
				Hold	95	10:00
				Cycle (40 Cycles)	95	0:15
					60	1:00
	Fast	48/96-well Fast	• Rxn. Volume: 20 µL • Ramp Speed: Fast	Hold§	50	2:00
				Hold	95	0:20
				Cycle (40 Cycles)	95	0:01
					60	0:20

‡ The 7300 system has only one run mode (Standard 7300).
§ Required for optimal AmpErase® UNG activity; not needed when UNG is not in the reaction.

For Research Use Only. Not for use in diagnostic procedures.

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