

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 [‡]
20X TaqMan® Pri-miRNA Assays	1.0	5.0
2X 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 (2X), 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 (2X), 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.

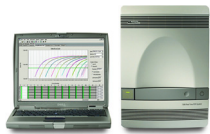
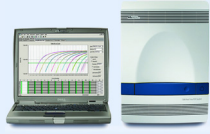


4 Run the plate

- 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Rxn. Volume: 20 µL Ramp Rate: Standard 	Hold [§]	50	2:00	
		384-well standard		<ul style="list-style-type: none"> Rxn. Volume: 20 µL Ramp Rate: Standard 	Hold	95	10:00
					Cycle (40 Cycles)	95	0:15
		60		1:00			
	Fast	96-well Fast	<ul style="list-style-type: none"> Rxn. Volume: 20 µL Ramp Rate: Fast 	Hold [§]	50	2:00	
				Hold	95	0:20	
		384-well standard		<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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.

Practice of the patented 5' Nuclease Process requires a license from Applied Biosystems. The purchase of the TaqMan Pri-miRNA Assays includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research when used with the separate purchase of an Authorized 5' Nuclease Core Kit. No other patent rights are conveyed expressly, by implication, or by estoppel. For further information on purchasing licenses contact the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

NOTICE TO PURCHASER: PLEASE REFER TO THE TAQMAN® PRI-miRNA ASSAYS PRODUCT INSERT AND PROTOCOL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

Trademarks of Life Technologies Corporation and its affiliated companies: Applied Biosystems®, AB (Design)®, StepOne™, and StepOnePlus™. AmpErase and TaqMan are registered trademarks of Roche Molecular Systems, Inc. All other trademarks are the sole property of their respective owners.

© Copyright 2009, Life Technologies Corporation. All rights reserved.

Part Number 4427720 Rev. B 05/2009

Headquarters

850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.345.5224
www.appliedbiosystems.com

International Sales

For our office locations please call the division headquarters or refer to our Web site at
www.appliedbiosystems.com/about/offices.cfm