

TaqMan® Gene Expression Assays

This Quick Reference Card provides instructions for using TaqMan® Gene Expression Assays and TaqMan® Non-coding RNA Assays. For detailed instructions, see the *TaqMan® Gene Expression Assays Protocol* (PN 4333458). For safety and biohazard guidelines, refer to the “Safety” section in the protocol. For all chemicals in **bold red** type, read the SDS 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! When using assays designed to a single exon (assay IDs with an _s or _g suffix), include DNase treatment in your RNA purification (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), pipet 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® Gene Expression Assay	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

[‡] (Optional) Use **TaqMan® Fast Advanced Master Mix** 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.

[#] Replicate volumes include 20% excess to compensate for volume loss from pipetting.

- 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.
- 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 for instructions on how to analyze your data.

Table 1 Plate document/experiment parameters for TaqMan® Gene Expression Assays - Standard Conditions

Applied Biosystems Real-Time PCR System	Reaction plate	Ramp Rate	Rxn. Volume	Thermal cycling conditions				
				Parameter	UNG Incubation [‡]	Polymerase Activation	PCR (40 Cycles)	
							Denature	Anneal/ Extend
Temp. (°C)	50	95	95	60				
ViiA™ 7 System	384-well standard	Standard [§]	20 µL	Time (mm:ss)	2:00	10:00	0:15	1:00
7900HT System	96-well standard							
	384-well standard							
StepOnePlus™/ StepOne™ System	48/96-well Fast							
7500 Fast System	96-well Fast							
7300/7500 System	96-well standard							

[‡] Required for optimal AmpErase® UNG activity; not needed when UNG is not in the reaction.

[§] The 7300 system has only one run mode (Standard 7300).

Table 2 Plate document/experiment parameters for TaqMan® Gene Expression Assay- Fast Conditions

Applied Biosystems Real-Time PCR System	Reaction plate	Ramp Rate	Rxn. Volume	Thermal cycling conditions				
				Parameter	UNG Incubation [‡]	Polymerase Activation	PCR (40 Cycles)	
							Denature	Anneal/ Extend
Temp. (°C)	50	95	95	60				
ViiA™ 7 System	384-well standard	Fast	20 µL	Time (mm:ss)	2:00	0:20	0:01	0:20
7900HT System	96-well Fast							
	384-well standard	Standard						
StepOnePlus™/ StepOne™ System	48/96-well Fast	Fast						
7500 Fast System	96-well Fast							
			2:00	0:20	0:03	0:30		

[‡] Required for optimal AmpErase® UNG activity; not needed when UNG is not in the reaction.

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