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[#]
20× TaqMan® Gene Expression Assay	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

[‡] (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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