

TaqMan® Gene Expression Master Mix

Quick Reference Card

For safety and biohazard guidelines, refer to the “Safety” section in the *TaqMan® Gene Expression Master Mix Protocol* (PN 4371135). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Gene Expression Procedure

This quick reference card provides simplified procedures for using the TaqMan® Gene Expression Master Mix with TaqMan® Gene Expression Assays. For details on performing real-time PCR, refer to the *TaqMan® Gene Expression Master Mix Protocol*.

STEP	ACTION																																					
1	Prepare the PCR reaction mix	<p>a. Prior to use, mix the TaqMan Gene Expression Master Mix thoroughly by swirling the bottle.</p> <p>b. Calculate the volume of each component needed for all the wells in each assay, based on the number of reactions. Include extra volume to compensate for the volume loss that occurs during pipetting.</p> <p>Note: Applied Biosystems recommends performing four replicates of each reaction.</p> <table border="1" data-bbox="518 864 1259 1298"> <thead> <tr> <th rowspan="3">Component</th> <th colspan="4">Simplex Reaction Volumes[†] (μL)</th> </tr> <tr> <th colspan="2">50-μL Reactions (Standard 96-Well Plate Setup)</th> <th colspan="2">20-μL Reactions (384-Well Plate or 96- or 48-Well Fast Plate Setup)</th> </tr> <tr> <th>Single Reaction</th> <th>Four Replicates with Excess[‡]</th> <th>Single Reaction</th> <th>Four Replicates with Excess[‡]</th> </tr> </thead> <tbody> <tr> <td>TaqMan Gene Expression Master Mix (2X)</td> <td>25.0</td> <td>125.0</td> <td>10.0</td> <td>50.0</td> </tr> <tr> <td>TaqMan Gene Expression Assay (20X)[§]</td> <td>2.5</td> <td>12.5</td> <td>1.0</td> <td>5.0</td> </tr> <tr> <td>cDNA template + H₂O^{††}</td> <td>22.5</td> <td>112.5</td> <td>9.0</td> <td>45.0</td> </tr> <tr> <td>Total Volume</td> <td>50.0</td> <td>250.0</td> <td>20.0</td> <td>100.0</td> </tr> </tbody> </table> <p>† For duplex reaction volumes, refer to the <i>TaqMan® Gene Expression Master Mix Protocol</i> (PN 4371135).</p> <p>‡ For every four reactions, include volume for a fifth reaction to provide excess volume for the loss that occurs during reagent transfers.</p> <p>§ See www.allgenes.com for TaqMan Gene Expression Assay information.</p> <p>†† For optimal performance of TaqMan Gene Expression Assays, use 10 to 100 ng of cDNA per 20- or 50-μL reaction, plus RNase-free water.</p> <p>c. Cap the tube(s).</p> <p>d. Vortex the tube(s) briefly to mix the solutions.</p> <p>e. Centrifuge the tube(s) briefly to spin down the contents and eliminate any air bubbles from the solutions.</p>				Component	Simplex Reaction Volumes [†] (μL)				50-μL Reactions (Standard 96-Well Plate Setup)		20-μL Reactions (384-Well Plate or 96- or 48-Well Fast Plate Setup)		Single Reaction	Four Replicates with Excess [‡]	Single Reaction	Four Replicates with Excess [‡]	TaqMan Gene Expression Master Mix (2X)	25.0	125.0	10.0	50.0	TaqMan Gene Expression Assay (20X) [§]	2.5	12.5	1.0	5.0	cDNA template + H ₂ O ^{††}	22.5	112.5	9.0	45.0	Total Volume	50.0	250.0	20.0	100.0
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2	Prepare the PCR reaction plate	<p>a. Transfer the appropriate volume of reaction mixture to each well of a plate:</p> <ul style="list-style-type: none"> • MicroAmp™ Fast Optical 48-Well Reaction Plate: 20 µL per well • MicroAmp™ Optical 96-Well Reaction Plate: 50 µL per well • MicroAmp™ Fast Optical 96-Well Reaction Plate: 20 µL per well • MicroAmp™ Optical 384-Well Reaction Plate: 20 µL per well <p>b. Cover the plate with MicroAmp™ Optical Adhesive Film. For MicroAmp™ 96-Well Reaction Plates, MicroAmp™ Optical Caps may be used instead.</p> <p>IMPORTANT! Use a MicroAmp Optical Film Compression Pad with Optical Adhesive Film when using a MicroAmp™ 96-Well Reaction Plate on the ABI PRISM® 7000 Sequence Detection System or the 7900HT Fast Real-Time PCR System.</p> <p>c. Centrifuge the plate briefly.</p>																			
3	Run the PCR reaction plate	<p>a. Set the thermal cycling conditions as follows:</p> <table border="1"> <thead> <tr> <th>Step</th> <th>Temperature (°C)</th> <th>Duration</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>UDG Incubation</td> <td>50</td> <td>2 min</td> <td>HOLD</td> </tr> <tr> <td>AmpliTaq Gold®, UP Enzyme Activation</td> <td>95</td> <td>10 min</td> <td>HOLD</td> </tr> <tr> <td>Denature</td> <td>95</td> <td>15 sec</td> <td rowspan="2">40</td> </tr> <tr> <td>Anneal/Extend</td> <td>60</td> <td>1 min</td> </tr> </tbody> </table> <p>b. In the plate document, select Standard mode and enter the correct sample volume (20 µL or 50 µL).</p> <p>IMPORTANT! Fast thermal cycling conditions are not for use with the TaqMan Gene Expression Master Mix. For information, refer to the <i>TaqMan® Gene Expression Master Mix Protocol</i>.</p>	Step	Temperature (°C)	Duration	Cycles	UDG Incubation	50	2 min	HOLD	AmpliTaq Gold®, UP Enzyme Activation	95	10 min	HOLD	Denature	95	15 sec	40	Anneal/Extend	60	1 min
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4	Analyze the results	Data analysis varies depending on the instrument. Refer to the <i>TaqMan® Gene Expression Master Mix Protocol</i> (PN 4371135) and your instrument user guide for information on how to review and adjust threshold values and other details on analyzing your data.																			

TaqMan Gene Expression Master Mix Products

Item	Part Number	Contents	Item	Part Number	Contents
Mini-Pack	4370048	One 1 mL tube (40 × 50 µL reactions)	5-Pack	4369510	5 × 5 mL bottles (1000 × 50 µL reactions)
1-Pack	4369016	One 5 mL bottle (200 × 50 µL reactions)	10-Pack	4369542	10 × 5 mL bottles (2000 × 50 µL reactions)
2-Pack	4369514	2 × 5 mL bottles (400 × 50 µL reactions)	Bulk Pack	4370074	One 50 mL bottle (2000 × 50 µL reactions)

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