TaqMan® Genotyping Master Mix

Quick Reference Card

For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® Genotyping Master Mix Protocol* (PN 4371131). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Genotyping Procedure

This quick reference card provides simplified procedures for using the TaqMan® Genotyping Master Mix with TaqMan genotyping assays. For details, refer to the *TaqMan Genotyping Master Mix Protocol*.

STEP	ACTION	
1	Prepare the PCR reaction mix	a. Prepare at least two no template controls (NTCs) and (if needed) at least one genomic DNA control of known genotype on each plate to ensure accurate genotype calling. 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.

PCR Reaction Mix Volumes‡ (µL/Well)

Component	Wet DNA Delivery Method			DNA Predelivery and Dry-Down Method		
	5-µL§	10-µL#	25-µL‡‡	5-µL§	10-µL#	25-µL‡‡
TaqMan Genotyping Master Mix (2X)	2.50	5.0	12.50	2.50	5.0	12.50
TaqMan genotyping assay mix (20X)	0.25	0.5	1.25	0.25	0.5	1.25
DNase-free, RNase-free water	(none)	(none)	(none)	2.25	4.5	11.25
Total Volume	2.75	5.5	13.75	5.0	10.0	25.00

‡ Example calculations of final volumes for ninety-six 25-µL reactions for the dry-down and predelivery method, with 14 extra reaction volumes:
-TaqMan Genotyping Master Mix: (12.5 µL × 110) = 1375 µL
-TaqMan genotyping assay mix: (1.25 µL × 110) = 137.5 µL
-DNase-free, RNase-free water: (11.25 µL × 110) = 1237.5 µL
§ Use with Optical 384-Well Reaction Plates.
# Use with Fast Optical 96-Well Reaction Plates. Refer to the *TaqMan®* Genotyping Master Mix *Protocol.*
‡‡ Use with standard Optical 96-Well Reaction Plates.

<table>
<tr><th>STEP</th><th colspan="2">ACTION</th></tr>
<tr><td>2</td><td>Prepare the reaction plate</td><td>
• If you use the DNA dry-down method:
a. In each well, pipette one control or sample (1 to 10 ng of purified genomic DNA).
b. Dry down the samples completely by evaporation at room temperature in a dark, amplicon-free location.
c. Transfer the appropriate volume of PCR reaction mix into each well:
• 5 µL per well for 384-well plates
• 10 µL per well for Fast 96-well plates
• 25 µL per well for 96-well plates
• If you use the wet DNA delivery method:
a. Transfer the appropriate volume of PCR reaction mix into each well:
• 2.75 µL per well for 384-well plates
• 5.5 µL per well for Fast 96-well plates
• 13.75 µL per well for 96-well plates
b. In each well, pipette one purified genomic DNA sample or control, diluted with DNase-free water to deliver a final DNA mass of 1 to 10 ng in a total volume of:
• 2.25 µL per well for 384-well plates
• 4.5 µL per well for Fast 96-well plates
• 11.25 µL per well for 96-well plates
</td></tr>
<tr><td>3</td><td>Run the PCR reaction plate</td><td>
a. Set the thermal cycling conditions as follows:
<table>
<tr><th>Step</th><th>Temperature (°C)</th><th>Duration</th><th>Cycles</th></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>
</table>
b. In the plate document, verify that the Fast thermal cycling mode is not selected, then enter the correct total sample volume (5 µL, 10 µL, or 25 µL).
IMPORTANT! Fast thermal cycling conditions are not for use with TaqMan Genotyping Master Mix. For information refer to the TaqMan Genotyping Master Mix Protocol.
</td></tr>
<tr><td>4</td><td>Read and analyze the results</td><td>Perform an endpoint plate read and analyze the results using an Applied Biosystems Real-Time PCR System. Refer to your instrument user guide for details on analyzing your data.</td></tr>
</table>

TaqMan Genotyping Master Mix Products

Item	Part Number	Contents
1-Pack	4371355	One 10-mL bottle
2-Pack	4381656	Two 10-mL bottles

Item	Part Number	Contents
Single Bulk Pack	4371357	One 50-mL bottle
Multi Bulk Pack	4381657	Two 50-mL bottles

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