

Power SYBR[®] Green PCR Master Mix: Performing Real-Time PCR Assays

Quick Reference Card

For safety and biohazard guidelines, refer to the “Safety” section in the *Power SYBR[®] Green PCR Master Mix and RT-PCR Protocol* (PN 4367218). For all chemicals in **bold red** type below, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference card provides simplified procedures for using the Power SYBR[®] Green PCR Master Mix for real-time PCR assays. The *Power SYBR[®] Green PCR Master Mix and RT-PCR Protocol* (PN 4367218) provides detailed real-time PCR and RT-PCR procedures and ordering information for the Power SYBR Green products.

STEP	ACTION																																									
1	Prepare the PCR master mix	a. Allow the Power SYBR [®] Green PCR Master Mix to thaw completely. b. In a polypropylene tube, prepare the PCR master mix by scaling the volumes listed below to the desired number of PCR reactions. Note: Include extra volume to account for pipetting losses. <table border="1" data-bbox="446 953 1252 1361" style="margin: 10px auto;"> <thead> <tr> <th data-bbox="446 953 657 1062" rowspan="2">Reaction Component</th> <th colspan="3" data-bbox="661 953 1087 991">Volume (μL) / Reaction</th> <th data-bbox="1091 953 1252 1062" rowspan="2">Final Concentration</th> </tr> <tr> <th data-bbox="661 996 787 1062">384-Well Plate</th> <th data-bbox="791 996 917 1062">96-Well Fast Plate[†]</th> <th data-bbox="921 996 1087 1062">96-Well Standard Plate</th> </tr> </thead> <tbody> <tr> <td data-bbox="446 1067 657 1147">Power SYBR Green PCR Master Mix (2X)</td> <td data-bbox="661 1067 787 1147" style="text-align: center;">5</td> <td data-bbox="791 1067 917 1147" style="text-align: center;">10</td> <td data-bbox="921 1067 1087 1147" style="text-align: center;">25</td> <td data-bbox="1091 1067 1252 1147" style="text-align: center;">1X</td> </tr> <tr> <td data-bbox="446 1152 657 1182">Reverse primer</td> <td data-bbox="661 1152 787 1182" style="text-align: center;">Variable</td> <td data-bbox="791 1152 917 1182" style="text-align: center;">Variable</td> <td data-bbox="921 1152 1087 1182" style="text-align: center;">Variable</td> <td data-bbox="1091 1152 1252 1182" style="text-align: center;">50 to 900 nM</td> </tr> <tr> <td data-bbox="446 1187 657 1216">Forward primer</td> <td data-bbox="661 1187 787 1216" style="text-align: center;">Variable</td> <td data-bbox="791 1187 917 1216" style="text-align: center;">Variable</td> <td data-bbox="921 1187 1087 1216" style="text-align: center;">Variable</td> <td data-bbox="1091 1187 1252 1216" style="text-align: center;">50 to 900 nM</td> </tr> <tr> <td data-bbox="446 1222 657 1251">Template</td> <td data-bbox="661 1222 787 1251" style="text-align: center;">Variable</td> <td data-bbox="791 1222 917 1251" style="text-align: center;">Variable</td> <td data-bbox="921 1222 1087 1251" style="text-align: center;">Variable</td> <td data-bbox="1091 1222 1252 1251" style="text-align: center;">1 to 100 ng</td> </tr> <tr> <td data-bbox="446 1256 657 1315">Nuclease-free water</td> <td data-bbox="661 1256 787 1315" style="text-align: center;">Variable</td> <td data-bbox="791 1256 917 1315" style="text-align: center;">Variable</td> <td data-bbox="921 1256 1087 1315" style="text-align: center;">Variable</td> <td data-bbox="1091 1256 1252 1315" style="text-align: center;">—</td> </tr> <tr> <td data-bbox="446 1321 657 1361">Total Volume</td> <td data-bbox="661 1321 787 1361" style="text-align: center;">10</td> <td data-bbox="791 1321 917 1361" style="text-align: center;">20</td> <td data-bbox="921 1321 1087 1361" style="text-align: center;">50</td> <td data-bbox="1091 1321 1252 1361" style="text-align: center;">—</td> </tr> </tbody> </table> <p data-bbox="446 1373 1252 1430" style="margin-top: 10px;"> [†] Assays performed using the Power SYBR[®] Green PCR Master Mix are not supported using Fast mode; therefore Optical 96-Well Fast Thermal Cycling Plates must be run under standard conditions. </p> c. Mix gently. <i>Do not vortex</i> . Centrifuge briefly, then prepare the PCR reaction plate.			Reaction Component	Volume (μL) / Reaction			Final Concentration	384-Well Plate	96-Well Fast Plate [†]	96-Well Standard Plate	Power SYBR Green PCR Master Mix (2X)	5	10	25	1X	Reverse primer	Variable	Variable	Variable	50 to 900 nM	Forward primer	Variable	Variable	Variable	50 to 900 nM	Template	Variable	Variable	Variable	1 to 100 ng	Nuclease-free water	Variable	Variable	Variable	—	Total Volume	10	20	50	—
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(continued on reverse)

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3	Run the PCR reaction plate	In the SDS software, open the plate document that corresponds to the reaction plate. Load the reaction plate into the instrument, then start the run. See your instrument user's manual for detailed instructions on how to load and run the plate.																											
4	Analyze the results	Data analysis varies depending on the instrument. See the <i>Power SYBR[®] Green PCR Master Mix and RT-PCR Protocol</i> (PN 4367218) and your instrument user's manual for detailed instructions on how to analyze your data.																											

† For optimal results, Applied Biosystems recommends using the 9600 Emulation mode. However, using the Standard run mode with the Power SYBR[®] Green PCR Master Mix provides comparable results. See the troubleshooting section of your instrument user's manual if you encounter poor performance.
‡ When Standard mode is selected, the Sequence Detection System Software (SDS Software) matches the ramp rate of the 9700 thermal cycler for standard PCR reactions.
§ When 9600 Emulation mode is selected, the SDS Software matches the ramp rate of the 9600 thermal cycler.

Power SYBR[®] Green PCR Master Mix Products

Item	Part Number	Contents
Power SYBR [®] Green PCR Master Mix:		
• Mini-Pack	• 4368577	• One 1 mL tube (40 × 50 µL reactions)
• 1-Pack	• 4367659	• One 5 mL tube (200 × 50 µL reactions)
• Bulk Pack	• 4367660	• One 50 mL tube (2000 × 50 µL reactions)
• 2-Pack	• 4368706	• 2 × 5 mL tubes (400 × 50 µL reactions)
• 5-Pack	• 4368702	• 5 × 5 mL tubes (1000 × 50 µL reactions)
• 10-Pack	• 4368708	• 10 × 5 mL tubes (2000 × 50 µL reactions)
Power SYBR [®] Green RT-PCR Reagents Kit	4368711	• Power SYBR [®] Green PCR Master Mix (200 × 50 µL reactions) • TaqMan [®] Reverse Transcription Reagents (200 × 10 µL reactions)
Related Documentation:		
• Protocol	• 4367218	• —
• Quick Reference Card	• 4367219	• —

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Notice to Purchaser: Please refer to the *Power SYBR[®] Green PCR Master Mix and RT-PCR Protocol* (PN 4367218) for limited label license or disclaimer information.

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