Power SYBR® Green PCR Master Mix: Executed on Roche LightCycler® 480 Real-Time PCR System

For safety and biohazard guidelines, refer to the "Safety" section in the *Power* SYBR® Green PCR Master Mix and RT-PCR Protocol (PN 4367218). 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 on the LightCycler® 480 Real-Time PCR System. 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 PCR Master Mix.

1 Prepare the PCR reagents mix

a. Allow the *Power* SYBR Green PCR Master Mix to thaw completely. Mix gently.
b. In a polypropylene tube, prepare the PCR reagents mix by scaling the volumes listed below to the desired number of PCR reactions.

Note: Include extra volume to account for pipetting losses.

Reaction Component	20 µL/Reaction	Final Concentration
Power SYBR Green PCR Master Mix (2X)	10	1X
Reverse Primer	Variable	50 to 300 nM
Forward Primer	Variable	50 to 300 nM
Template	Variable	1 to 100 ng
Nuclease-free water	Variable	-

c. Mix gently. Do not vortex. Centrifuge briefly and then prepare the PCR reaction plate.

2 Set up the plate document

See your instrument user's manual for detailed instructions on how to configure the plate documents.
The thermal-cycling condition for the *Power* SYBR Green PCR Master Mix are described in the table below:

Step	AmpliTaq Gold® Enzyme Activation (*)	PCR	
	Hold	Cycles (40 cycles)	
		Denature	Anneal/Extend (**)
Time	10 min	15 sec	60 sec
Temp (°C)	95	95	60

Another option is to download the template "ABPowerSYBRThermalProtocolLC480" from www.appliedbiosystems.com. To use this template, open the software and follow the steps below:

a. Click on "Navigator" button, select the template folder, click "Import" and select the file "ABPowerSYBRThermalProtocolLC480". Click "open".

b. Click on "save" button to save the template in the template folder. Click "yes".

c. To create a new plate, click on "overview" button, click on "new experiment" and click on "Apply Template". Select the "ABPowerSYBRThermalProtocolLC480" template***.

d. Finish to set-up the plate (sample name, replicate…)

Note:

* Never decrease the temperature or time for the AmpliTaq Gold® Enzyme Activation.

** Before setup, check your primer annealing temperature. If primer Tm is < 60°C, we recommend using a 3-step protocol.

*** ROX™ is included in the *Power* SYBR Green PCR Master Mix. LightCycler® 480 Real-Time PCR System can detect this dye but it will not be used for fluorescence normalization in the LightCycler® 480 Real-Time PCR System software.

3	Run the PCR reaction plate	Load the reaction plate into the instrument, then click "Start Run" tab to 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 *Power* SYBR® Green PCR Master Mix and RT-PCR Protocol (PN 4367218) and your instrument user's manual for detailed instructions on how to analyze the data.

Power SYBR® Green PCR Master Mix Products

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

± Based on 50 µL reaction volume.

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