Fast SYBR® Green Master Mix:
Executed on Bio-Rad CFX96 Real-Time PCR Detection System

For safety and biohazard guidelines, refer to the "Safety" section in the Fast SYBR® Green Master Mix Protocol (PN 4385372). 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 Fast SYBR Green Master Mix for real-time PCR assays on the CFX96 Real-Time PCR Detection System. The Fast SYBR Green Master Mix Protocol (PN 4385372) provides detailed real-time PCR and RT-PCR procedures and ordering information for the Fast SYBR Green Master Mix.

1 Prepare the PCR reagents mix

a. Allow the Fast SYBR Green 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
Fast SYBR Green 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 Fast SYBR Green Master Mix are described in the table below:

Step	Enzyme Activation	PCR	
	Hold	Cycles (40 cycles)	
		Denature	Anneal/Extend (*)
Time	20 sec	3 sec	30 sec
Temp (°C)	95	95	60

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

a. Under "File", select "New" then "Experiment".

b. Under "Protocol" tab, click "Select Existing". Choose the protocol file named "ABFastSYBRThermalProtocolCFX".

c. Click "edit" button to modify the template if needed (change the sample volume for example). Click "OK" to save the modification.

d. Under "Plate" tab, click "Select Existing". Choose a plate file named "Quick Plate_96 wells_SYBR Only.pltd". The instrument will only collect SYBR green dye fluorescence**.

Note:

* 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 Fast SYBR Green Master Mix, but will not be used for fluorescence normalization in the Bio-Rad CFX Manager 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 Fast SYBR® Green Master Mix Protocol (PN 4385372) and your instrument user's manual for detailed instructions on how to analyze the data.

Fast SYBR® Green Master Mix Products

Item	Part Number	Contents
Fast SYBR Green Master Mix ±		
• Mini-Pack	• 4385610	• 1 x 1 mL tube (100 reactions)
• 1-Pack	• 4385612	• 1 x 5 mL bottle (500 reactions)
• 2-Pack	• 4385616	• 2 x 5 mL bottles (1000 reactions)
• 5-Pack	• 4385617	• 5 x 5 mL bottles (2500 reactions)
• 10-Pack	• 4385618	• 10 x 5 mL bottles (5000 reactions)
• Bulk Pack	• 4385614	• 1 x 50 mL bottle (5000 reactions)
Related Documentation		
• Protocol	• 4385372	• 1 protocol
• Quick Reference Card	• 4385371	• 1 card

± Based on 20 µL reaction volume.

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