Fast SYBR™ Green Master Mix

Catalog Numbers 4385610, 4385612, 4385614, 4385616, 4385617, and 4385618

Pub. No. 4385371 **Rev.** C

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Fast SYBR™ Green Master Mix User Guide* (Pub. No. 4385372). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference provides simplified procedures for using the Applied Biosystems™ Fast SYBR™ Green Master Mix. For details on performing real-time PCR, refer to the *Fast SYBR™ Green Master Mix User Guide* (Pub. No. 4385372).

Contents and storage

Cat. No.	Number of 20-µL reactions	Amount	Storage
4385610	100	1 × 1 mL	2–8°C
4385612	500	1 × 5 mL	
4385616	1,000	2 × 5 mL	
4385617	2,500	5 × 5 mL	
4385618	5,000	10 × 5 mL	
4385614	5,000	1 × 50 mL	

Methods

1 Prepare the PCR reaction mix

a. Prior to use, mix the Fast SYBR™ Green Master Mix thoroughly by swirling the bottle.

b. Calculate the volume of each component needed for all the wells in each assay, based on the number of reactions.

Note: We recommend performing four replicates of each reaction.

Component	Volume for one reaction
Fast SYBR™ Green Master Mix (2X)	10 µL
Forward and reverse primers[1]	Variable
cDNA template + RNase-free water[2]	Variable
Total volume	20 µL

[1] For optimal performance, use a minimum of 200 nM of each primer.

[2] For optimal performance, use up to 20 ng of cDNA per 20-µL reaction.

c. Cap the tube(s).

d. Vortex the tube(s) briefly to mix the solutions.

e. Centrifuge the tube(s) briefly to spin down the contents and eliminate any air bubbles from the solutions.

2 Prepare the PCR reaction plate

a. Transfer the appropriate volume of reaction mixture to each well of a plate:

Plate type	Volume per well
MicroAmp™ Fast Optical 48-Well Reaction Plate	20 µL
MicroAmp™ Fast Optical 96-Well Reaction Plate	20 µL
MicroAmp™ Optical 384-Well Reaction Plate	20 µL

b. Seal the plate with an optical adhesive cover, then centrifuge the plate briefly.

3 Run the PCR reaction plate

a. Set the thermal cycling conditions as specified in the following table:

Select Fast Mode				
Instrument	**Step**	**Temperature**	**Duration**	**Cycles**
• QuantStudio™ 3, 5, 6 Flex, 7 Flex, and 12k Flex • ViiA™ 7 • StepOne™ • StepOnePlus™ • 7500 Fast	AmpliTaq™ Fast DNA Polymerase, UP activation	95°C	20 seconds	HOLD
	Denature	95°C	3 seconds	40
	Anneal/extend	60°C	30 seconds	
7900HT Fast	AmpliTaq™ Fast DNA Polymerase, UP activation	95°C	20 seconds	HOLD
	Denature	95°C	1 seconds	40
	Anneal/extend	60°C	20 seconds	

b. In the plate document, select the mode and enter the correct sample volume (20 µL).

4 Analyze the results

Data analysis varies depending on the instrument. Refer to the *Fast SYBR™ Green Master Mix User Guide* (Pub. No. 4385372) and your instrument user guide for information.

Limited product warranty

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Revision history: Pub. No. 4385371

Revision	Date	Description
C	4 May 2016	Added instruments to Step 3 Format, style, and legal updates
B	September 2007	Baseline for this revision history

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