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2X Fast qPCR Master Mix-SYBR Green

Product Name and Catalog Number

2X Fast qPCR Master Mix-SYBR Green
Cat. # W157-NR, W157-LR, W157-HR,
Size: 2 x 1ml for 200 reactions (No ROX, Low ROX or High ROX)

Intended Use

- This 2X Fast qPCR Master Mix is used for real-time qualitative and quantitative qPCR with SYBR Green dye. This product is for research use only.
- The master mix is a premixed, 2X concentrated solution that has all the components except for gene-specific primers and DNA template.

Kit Features

- The kit is designed for singleplex qPCR with SYBR Green dye.
- The kit contains *Taq*-Fast DNA polymerase which can extend more than 300 bases with a short cycling program.
- The concentrations of the primers are variable depending on assay designs and thermo-cycling protocols (Table 1).
- The preferable PCR product size is ≤150bp.
- The kit has three formulations of ROX, Low ROX or High ROX concentrations for your choice (see Table 2).

Kit Contents

2X Master Mix (2x1ml for 200 reactions)

Transportation and Storage

The kit can be transported at ≤4°C for up to 3 days.

The kit should be stored in the dark at -20°C for no more than one year with no more than 10 times of freeze-thaw cycles. The kit can be stored at ≤4°C for a week.

Table 1. Setting Up a 20μL or 10μL reaction

Component	Volume per 20μL	Volume per 10μL	Final concentration
2X Master Mix	10μL	5μL	1X
Primers[a]	Variable	Variable	Each 150-900nM
DNA template[b]	Variable	Variable	≤60ng or ≤30ng genomic DNA
H_2O	To 20μL	To 10μL	

Footnotes of Table 1

[a] Each primer's T_m should be designed ≥60˚C, preferably between 62˚C to 65˚C, using primer3 software for high efficiency and specificity.

[b] DNA templates should be extracted by a qualified silica-based kit and eluted with low EDTA TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0-8.3).

Applicable Instruments

Table 2. Compatible instruments

PCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler®	Not recommended	Not necessary
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye button
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye button

Setting Up Thermal Cycling

Table 3. Standard thermo-cycling Protocol

Stage	Temperature	Period	Number of cycles
I	95°C	2min	1
II	95°C	12sec	35-40
	60°C, signal acquisition	60sec	
III	60°C to 95°C	Various	1

Footnotes of Table 3

The primer concentration used is typically 0.2uM.

Table 4. Fast thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	95°C	1min	1
II	95°C	5sec	35-40
	60°C, signal acquisition	30sec	
III	60°C to 95°C	Various	1

Footnotes of Table 4

The product size for the fast thermocycling protocol is preferred to be less than 90bp.

The primer concentration used is typically between 0.4uM and 0.9uM.

Quality Control

Not detectable DNase and RNase contaminations.

Related Products

- 2X qPCR Master Mix-TaqMan probe, Cat.# W153 **(No ROX, High ROX or Low ROX)**
- 2X Multiplex qPCR Master Mix-TaqMan probe, Cat.# W156 **(No ROX, High ROX or Low ROX)**

Precautions

If you order a "**No ROX**" master mix but you have an Applied Biosystems/ThermoFisher instrument, please **turn off ROX passive reference dye button** when setup assays.