Research use only

2X Multiplex qPCR Master Mix-TaqMan Probe

Product Name and Catalog Number

2X Multiplex qPCR Master Mix-TaqMan Probe**,** Cat qPCR 156, 2x1mL/5x1mL/10mL for 200/500/1000 reactions (No ROX, Low ROX or High ROX)

Intended Use

- The 2X Multiplex qPCR Master Mix is used for real-time qualitative and quantitative multiplex qPCR with TaqMan probes for up to 4 targets.
- The master mix is a premixed, 2X concentrated solution that has all the components except for gene-specific primers, probes and templates

Kit Characterizations

- The kit is designed for multiplex qPCR with TaqMan probes.
- The kit uses *Taq*-Probe DNA polymerase specially engineered for TaqMan probes, which increased 5' to 3' exonuclease activity produces S-shaped curve.
- Two–four pairs of gene-specific primers can be applied in one reaction.
- The concentrations of the primers and probes are variable depending on specific assays and thermocycling 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/5x1mL/10mL for 200/500/1000 reactions) and an instruction for use.

Transportation and storage

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

The kit should be kept stable in the dark at -20°C for ≤24 months with ≤10 times of freeze-thaw cycles. The kit can be stored at 4°C for a weak.

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
TaqMan probes[b]	Variable	Variable	Each 150-250nM
DNA templates[c]	Variable	Variable	≤500ng human genomic DNA/20μL
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] Each probe's T_m should be 8-10°C higher than the primer's T_m, preferably between 70-75°C.

[c]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

qPCR 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 Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	95°C	2min	1
II	95°C	10sec	35-40
	60°C, signal acquisition	60sec	

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	

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 qPCR 153 **(No ROX, High ROX or Low ROX)**
- 2X Fast qPCR Master Mix-SYBR Green, Cat qPCR 157 **(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.