2x Multiplex qPCR Mastermix for TaqMan Probe (No ROX)

Cat. No.	Product Description	Size
QPTM01-00	2x Multiplex qPCR Mastermix for TaqMan probe	1 ml
QPTM01-01	2x Multiplex qPCR Mastermix for TaqMan probe	5x1 ml
QPTM01-02	2x Multiplex qPCR Mastermix for TaqMan probe	50 ml

Intended Use

- The 2x Multiplex qPCR Mastermix is used for real-time qualitative and quantitative multiplex qPCR with TaqMan probes for up to four targets.
- The Mastermix 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 Super *Taq*-Probe DNA polymerase specially engineered for TaqMan probes, which increases 5' to 3' exonuclease efficiency and produces S-shaped curve.
- Two to 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 No ROX, Low ROX or High ROX concentrations for your choice (see Table 2).

Transportation and storage

- The kit can be transported at below 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 week.

Setup Reaction and Thermocycling

1. Thaw 2x Mastermix and other reaction components at room temperature, mix each component, centrifuge and then place on ice.
2. Set up reactions (Table 1).
3. Seal tubes with flat, optically transparent caps or seal plate with optically transparent film.
4. Mix and then briefly centrifuge the tubes or plate.
5. Program PCR instrument with indicated thermo-cycling protocol.
6. Load PCR tubes or plate and start to run.
7. Perform data analysis according to the PCR instrument instructions.

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

Component	Volume per 20μL	Volume per 10μL	Final concentration
2x Mastermix	10μL	5μL	1X
Primers[a]	Variable	Variable	Each 150-400nM
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).

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
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye

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	

Table 4. Three-Step Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	95°C	2min	1
II	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal acquisition	30sec	

Footnotes of Tables 3 and 4

- The three-step thermocycling protocol in Table 4 increases overall polymerase activity by 50%, a more effective protocol than Table 3.
- The primer concentration used in Tables 3 and 4 is typically 0.15-0.2uM.

Related Products

- 2x qPCR Mastermix for TaqMan Probe
- 2x Fast qPCR Mastermix-SYBR Green

Precautions

If you order a "**No ROX**" Mastermix but you have an Applied Biosystems or Thermo Fisher instrument, please **turn off ROX passive reference dye button** when setup assays.