

# Multiplex Probe qPCR Mix Plus U (Low ROX+)

For research use only

## Cat. No./Spec.

Cat. No.	P2701a	P2702a	P2703a	P2704a
25- $\mu$ l rxn Nos	80 rxns	400 rxns	4000 rxns	8000 rxns

## Components

Component	P2701a	P2702a	P2703a	P2704a
2 $\times$ Multiplex Probe qPCR Mix Plus U (Low ROX+)	1 ml	1 ml $\times$ 5	50 ml	100 ml
Nuclease-free Water	1 ml	1 ml $\times$ 5	-	-

## Storage

This reagent can be stored for 2 months at 4°C. For longer storage, it should be kept at -20°C.

## Description

Multiplex Probe qPCR Mix Plus U (Low ROX+) is a 2 $\times$  concentrated premix for real-time quantitative PCR with probe method. In use, just add the DNA template, primer and probe to react. This product contains antibody technology modified Hotstart Taq DNA polymerase. Combined with GDSBio's special real-time PCR Buffer, it can not only effectively inhibit primers dimer and other non-specific amplification, but also improve the amplification efficiency, allowing multi-probe qPCR reaction. This reagent introduced dUTP/UDG anti-contamination system, which can remove PCR products containing dUTP before PCR reaction, effectively avoid the influence of cross contamination of amplification products on quantification. This product is convenient for use with TaqMan, primers to achieve a fully premixed Master Mix molecular diagnostic kit, and is perfectly compatible with common quantitative PCR instruments, such as ABI, Roche, Bio-Rad, etc. The product has been pre-mixed with low-concentration ROX reference dye, which is suitable for ABI, Roche and other fluorescent quantitative PCR instruments requiring low-concentration ROX for calibration. It can be used with TaqMan and other fluorescent probes.

The reaction system of this product can be prepared at room temperature without an ice box. The prepared PCR reaction system can be placed at room temperature for 24 hours and the amplification efficiency remains unchanged.

## Applications

- Probe gene expression analysis
- Probe Low-copy gene detection
- Probe microarray validation
- Probe gene knockdown validation

## Features

- This kit is suitable for fluorescence quantification by probe method

- This kit is compatible with many real-time systems
- Hot-start technology brings high specificity and reproducible amplification
- dUTP/UDG system, effectively prevent PCR product contamination

## Table of Instrument Guide

Instrument	Conc. of ROX
ABI PRISM 7000/ PRISM 7700/ 7300/ 7900HT/ Step One/ Step One Plus/ GeneAmp 5700	500 nM (High ROX)
ABI 7500/ 7500 Fast/ ViiA 7/ QuantStudio 6/7/12K Flex; Agilent Stratagene Mx3000P/ Mx3005P/ Mx4000	50 nM (Low ROX)
Bio-Rad CFX96/ CFX384/ iQ/ iQ5; MJ Research Opticon 2/ Chromo 4; Roche LightCycler 480/ 96; Corbett Rotor Gene G/ Q/ 3000/ 6000; Thermo PikoReal 96; Eppendorf MasterCycler ep realplex; Cepheid Smart Cyclor	No ROX

## Protocol

### 1. Preparation of reaction solution

Add the following reagents to the proper thermal cycler reaction tube or plate on ice:

Component	25- $\mu$ l rxn	Final Conc.
2 $\times$ Multiplex Probe qPCR Mix Plus U (Low ROX+)	12.5 $\mu$ l	1 $\times$
Forward Primer (10 $\mu$ M)	0.5 $\mu$ l	0.2 $\mu$ M
Reverse Primer (10 $\mu$ M)	0.5 $\mu$ l	0.2 $\mu$ M
Probe (10 $\mu$ M)	1 $\mu$ l	0.1~0.5 $\mu$ M
Template DNA	2 $\mu$ l	-
Water, nuclease-free	8.5 $\mu$ l	-

### Note:

- Prepare according to the recommended volume of each instrument.
- The optimal range for primers is 0.1~1.0  $\mu$ M. In general, the primers with a final concentration of 0.2  $\mu$ M work well.
- The concentration of the probe used is related to the Real Time PCR amplification instrument, probe species and types of fluorescent label. Please refer to the instructions when using it. Typically, the final concentration is between 0.1 and 0.5  $\mu$ M.
- Use 1-10ng cDNA or 10-100ng gDNA for each reaction of 25  $\mu$ l system.
- Users can increase the amount of the the qPCR Mix when using low-copy gene as template.

### 2. Setup the plate

Transfer the reaction mixture to PCR tubes/plates. Reaction volumes can be reduced to 10  $\mu$ l if the instrument supports a low volume system.

Cap or seal the reaction tubes/plates then centrifuge briefly to spin down the contents and eliminate any air bubbles.

### 3. Perform qPCR using the following thermal cycling condition

Set the thermal cycling conditions using default PCR thermal cycling conditions specified in the following tables according to the instrument cycling parameters of the specific primers.

#### 2-step PCR mode:

Stage	Temperature	Time	Cycle
UDG Pre-treatment	37°C	2 min	1
Initial Denaturation	95°C	30 sec	1
Denaturation	95°C	10 sec	40
Annealing & Extension*	60°C	30 sec	

\*The instrument do signal acquisition at the Annealing & Extension stage, and some instruments require more than 30 seconds, such as ABI 7300 for at least 31 seconds and ABI 7500 for at least 34 seconds.

If the reaction performs not well, it is recommended to adopt a 3-step amplification procedure.

#### Standard 3-step PCR mode:

Stage	Temperature	Time	Cycle
UDG Pre-treatment	37°C	2 min	1
Initial Denaturation	95°C	30 sec	1
Denaturation	95°C	10 sec	40
Annealing*	60°C	15 sec	
Extension	72°C	20 sec	

\*The instrument do signal acquisition at this stage. The usual annealing temperature is 55-65 °C. The annealing temperature is generally set to  $T_m - 5$  °C of the primer used, generally not less than 55 °C. (melting temperature,  $T_m$ ). Set the time of annealing according to the instrument guide.

### 4. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

#### Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

#### Product Use Limitations

This product is sold exclusively for research purposes and in vitro use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.