Cat. No: P2601, 66 rxn/30μl reaction P2602, 330 rxn/30μl reaction
P2603, 3333 rxn/30μl reaction P2604, 6666 rxn/30μl reaction

Multiplex Probe qPCR Mix

For research use only

Components

Component	P2601	P2602	P2603	P2604
2× Multiplex Probe qPCR Mix[a]	1 ml	1 ml × 5	50 ml	100 ml
Nuclease-free Water	1 ml	1 ml × 5	-	-

a contains Hotstart Taq DNA Polymerase, dNTP mix and reaction buffer.

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 is a 2× 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 product is convenient for preparation of IVD molecular diagnostic kit. The target gene quantification is accurate, reliable and reproducible. This product can be used with TaqMan and other fluorescent probes, and is perfectly compatible with common quantitative PCR instruments, such as ABI, Roche, Bio-rad, etc.

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

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 Cycler	No ROX

Protocol

This protocol is intended for use without ROX reference dye. Customers need to prepare ROX Reference Dye according to the table **"Table of Instrument Guide"**, if the instrument needs them.

1. Preparation of reaction solution

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

Component	Volume	Final concentration
2× Multiplex Probe qPCR Mix	15 μl	1×
Forward Primer (10μM)	0.6 μl	0.2 μM
Reverse Primer (10μM)	0.6 μl	0.2 μM
Probe (10μM)	0.3 μl	0.1 μM
Template DNA	2~5 μl	1~100ng/30 μl
Water, nuclease-free	to 30 μl	–

Note:

- Prepare according to the recommended volume of each instrument.
- The optimal range for primers is 0.1~1.0 μM. In general, the primers with a final concentration of 0.2 μM 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 μM.
- Use 1-10ng cDNA or 10-100ng gDNA for each reaction of 30 μl 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 μl 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

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
Initial Denaturation	95ºC	30 sec	1
Denaturation	95ºC	10 sec	40
Annealing*	60ºC	15 sec	
Extension	72ºC	20 sec	

*The instrument do signal acquisition at this stage. The usual annealing temperature is 55-65 ºC. The annealing temperature is generally set to Tm-5 ºC of the primer used, generally not less than 55 ºC. (melting temperature, Tm). 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.

Important Notes

Assay Design

We recommend using previously validated assays or using dedicated qPCR design software such as Beacon Designer 7 when designing Probe-based assays (www.PremierBiosoft.com).

Lyophilized primers and probes should be resuspended in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. DNA kept frozen in a nuclease-free environment should be stable for years. We find it convenient to initially prepare a 100 µM freezer stock (which should be thawed relatively infrequently).

Optimal primer concentration should be determined empirically. To maximize the sensitivity of the assay, use the lowest concentration of primers that can be used without compromising the efficiency of the qPCR reaction. The optimal primer concentration range is 100-400 nM.

Optimal probe concentration should be determined empirically. The optimal probe concentration range has generally been found to be 0.1-0.5 µM.

Template

Genomic DNA, plasmid DNA, or cDNA can be used as template. For optimal quantitative results use up to 20ng of genomic DNA or plasmid DNA per 20 µl reaction (for smaller volumes, the amount of template should be decreased equivalently). Using greater amounts of template may reduce the maximum fluorescence signal due to binding of the probe to the template. For two-step RT-PCR, use either undiluted or diluted cDNA generated from up to 1µg of total RNA. The volume of the cDNA added (from the RT reaction) should not exceed 10% of the final PCR volume (e.g., for a 20 µl qPCR reaction, use up to 2.0 µl of undiluted cDNA).

Primers

Careful primer design and purification (HPLC-purified primers are recommended) is particularly important in order to minimize loss in sensitivity due to the production of nonspecific amplification products in qPCR. This effect becomes more prominent at low target concentrations. To maximize the sensitivity of the assay, use the lowest concentration of primers that can be used without compromising the efficiency of the PCR reaction (0.1-1.0 µM of each primer). For optimal results, design primers that amplify PCR products 50-150 bp in length. The primers should exhibit a melting temperature (Tm) of approximately 60ºC. We recommend designing primers specifically for amplification of cDNA derived from mRNA. This prevents amplification of contaminating genomic DNA and inaccurate quantification of mRNA.

Magnesium chloride

The concentration of $MgCl_2$ affects the binding dynamics of primers and probes to template DNA. The higher the final $MgCl_2$ concentration in the PCR reaction, the greater the binding affinity of the primers and probe for target DNA. 2× Multiplex Probe qPCR Mix provides $MgCl_2$ at a final concentration which is suitable for most targets.

Guidelines for preventing contamination of qPCR reaction

During qPCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

• Prepare your DNA sample, set up the qPCR mixture, perform thermal cycling and analyze PCR products in separate areas.

• Set up a PCR mixtures in a laminar flow cabinet equipped with an UV lamp.

• Wear fresh gloves for DNA purification and reaction set up.

• Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.

• Always perform “no template control” (NTC) reactions to check for contamination

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucl- eases 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.