

Cat. No: P2201, 100 rxns/20µl reaction
P2202, 500 rxns/20µl reaction

HS Probe qPCR Mix

For research use only

Components

| Component | P2201 | P2202 |
|----------------------|-------|----------|
| 2× HS Probe qPCR Mix | 1 ml | 1 ml × 5 |
| Nuclease-free Water | 1 ml | 1 ml × 5 |

Storage

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

Description

HS Probe qPCR Mix is designed for high-performance, high-throughput real-time PCR using sequence-specific fluorogenic probes. 2× HS Probe qPCR Mix contains the components (except primers, DNA template and probe) that necessary to perform PCR. The core component of the mix is Optimus™ Hotstart Taq DNA Polymerase, a hot-start polymerase, which brings high specificity by reducing non-specific products as the enzyme activity is temperature-dependent and is inhibited at room temperature. Combined with well-optimized buffer can significantly improve amplification efficiency. This product 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

Composition of the 2× HS Probe qPCR Mix

KCl, MgCl₂, dNTPs, Optimus™ Hotstart Taq DNA Polymerase, and other optimized buffer components.

Table of Instrument Guide

| Instrument | Conc. of ROX (100×) |
|------------|---------------------|
|------------|---------------------|

| | |
|--|----------|
| ABI® PRISM® 7000, 7700, 7900HT, ABI® 7300 qPCR Systems, GeneAmp® 5700, StepOne™, and the StepOnePlus™ | 1-2% ROX |
| ABI® 7500 qPCR Systems, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™ Mx3005P™ and Mx4000™ | 0.2% ROX |
| BioRad iCycler MiniOpticon, Opticon 2, Chromo 4, iQ5; Roche LightCycler 480; MJ Research DNA Engine Opticon 2, Chromo 4; Corbett Rotogene 3000, 6000 | No ROX |

Protocol

This protocol is intended for use with the BioRad iCycler MiniOpticon, Opticon 2, Chromo 4, iQ5; Roche LightCycler 480; MJ Research DNA Engine Opticon 2, Chromo 4; Corbett Rotogene 3000, 6000. Customers need to prepare ROX Reference Dye, 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× HS Probe qPCR Mix | 10 µl | 1× |
| Forward Primer (10µM) | 0.4 µl | 0.2 µM |
| Reverse Primer (10µM) | 0.4 µl | 0.2 µM |
| Probe (10µM) | variable | 0.1-0.5 µM |
| Template DNA | variable | 0.05-5 ng/µl |
| Water, nuclease-free | to 20 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 20 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. Preform 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.

Standard 3-step PCR mode:

| | | | |
|----------------------|------|--------|----------------------------|
| Initial Denaturation | 95°C | 3 min | Holding Stage |
| Denaturation | 95°C | 10 sec | Cycling Stage 40 Cycles |
| 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.

2-step PCR mode:

| | | | |
|------------------------|------|--------|----------------------------|
| Initial Denaturation | 95°C | 3 min | Holding Stage |
| Denaturation | 95°C | 10 sec | Cycling Stage 40 Cycles |
| Annealing & Extension* | 60°C | 30 sec | |

*The instrument do signal acquisition at the 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.

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 (T_m) of approximately 60°C. 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 MgCl₂ affects the binding dynamics of primers and probes to template DNA. The higher the final MgCl₂ concentration in the PCR reaction, the greater the binding affinity of the primers and probe for target DNA. 2× HS Probe qPCR Mix provides MgCl₂ 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, 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

HS Probe qPCR Mix 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.