

Pro Multiplex Probe qPCR Mix

For research use only

Cat. No./Spec.

Cat. No.	P2731	P2732	P2733	P2734
25- μ l rxn Nos.	80 rxns	400 rxns	4000 rxns	8000 rxns

Components

Component	P2731	P2732	P2733	P2734
2X Pro Multiplex Probe qPCR Mix	1 ml	1 ml $\times$ 5	50 ml	100 ml

Storage

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

Description

Pro Multiplex Probe qPCR Mix is a 2X Master Mix designed for multiplex real-time quantitative PCR using probe-based detection. The reaction can be performed simply by adding template, primers, and probes. This product contains a mutant hot-start enzyme, Hotstart Taq DNA polymerase, modified with antibody technology, combined with Dongsheng's specially formulated PCR Buffer, enabling highly sensitive multiplex qPCR. The reagent incorporates a dUTP/UDG contamination prevention system, which can eliminate dUTP-containing PCR products before amplification, effectively avoiding quantitative interference caused by cross-contamination of amplification products. This product is convenient for developing fully pre-mixed Master Mix molecular diagnostic kits with probes and primers. It is compatible with various fluorescent probes such as TaqMan and works seamlessly with common quantitative PCR instruments from manufacturers like ABI, Roche, and Bio-Rad. This product allows reaction setup without an ice box—the PCR Mix, primers, and template can be prepared at room temperature, and the prepared PCR reaction system can be stored at room temperature for 24 hours without loss of amplification efficiency.

Applications

- Probe gene expression analysis
- Probe Low-copy gene detection
- Multiplex probe qPCR
- IVD diagnostic kit research

Features

- Ready-to-use 2X Mix for multiplex qPCR

- High sensitivity and multiplex capability
- Built-in contamination control (dUTP/UDG)
- Broad probe and instrument compatibility
- Stable at room temperature for 24 hours

Protocol

1. Preparation of reaction solution

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

Component	25- μ l rxn	Final Conc.
2X Pro Multiplex Probe qPCR Mix	12.5 μ l	1X
Forward Primer (10 μ M)	0.5 μ l	0.2 μ M
Reverse Primer (10 μ M)	0.5 μ l	0.2 μ M
Probe (10 μ M)	1 μ l	0.1~0.5 μ M
Template DNA	2 μ l	-
Water, nuclease-free	8.5 μ l	-

Note:

- The optimal range for primers is 0.1~1.0 μ M. In general, the primers with a final concentration of 0.2 μ 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 μ M.
- For certain instrument models, the addition of ROX reference dye is required to accurately determine the Ct value. Due to the small volume of ROX used, it is recommended to pre-mix the ROX with the qPCR Mix before use. Refer to the specific instrument manual for the required amount of ROX.

2. Setup the plate

Transfer the reaction mixture to PCR tubes/plates. Reaction volumes can be reduced to 10 μ 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.*

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.