TOOLS Easy 2xProbe qPCR Mix

Cat. no. TTC-QE13
Storage: -20℃ protected from light for 1 year
Product Size: 1.25 ml (125 rnx/20 ul reaction)

Introduction

TOOLS Easy 2×Probe qPCR Mix is suitable for real-time quantitative polymerase chain reaction (PCR) assays. This ready-to-load mix contains dNTP/dUTP mix, Mg^{2+}, DNA polymerase, dUTP/UDG anticontamination system, specific ROX Reference Dye, and all components necessary to perform qPCR, except templates, primers, and probes. The polymerase in this kit is a chemically modified hot-start DNA polymerase. The kit contains optimized buffer that effectively inhibits nonspecific amplification and significantly improves the efficiency of qPCR reactions. The dUTP/UDG anticontamination system added to the mix eliminates the product contamination of qPCR reactions. The 2× reaction mix can be directly used for a robust and low-template qPCR with high sensitivity, specificity, and reliability. TOOLS Easy 2×Probe qPCR Mix also contains ROX Reference Dye suitable for all qPCR instruments, and no adjustments are required for the dye concentration in different instruments.

Compatible devices:

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus™ 7500, 7500 Fast, ViiA™7;

Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000;

Stratagene MX4000™, MX3005P™, MX3000P™;

Eppendorf Mastercycler® ep realplex, realplex 2 s;

Roche Applied Science LightCycler™ 480 and other instruments;

Materials not supplied

1. Optical-grade qPCR tubes, plates, sealing films, and aerosol-resistant pipette tips.
2. qPCR primers and probes.
3. DNA or cDNA templates.

Protocol

For qPCR setup:

1. The final concentration of the primer should be set as 0.2 μM for most applications. The primer concentration can be adjusted from 0.1 to 1.0 μM for different experiments.
2. The final concentration of the probe should be in the range of 50–250 nM, and the Probe labeled by ROX should not be used.
3. The length of amplified PCR products should be in the range of 70–200 bp.
4. A dilution series of templates should be prepared to assess the standard curve and test primer efficiency.
5. Approximately 1–50 ng of DNA template should be used for a 20-μL reaction. If the template is a stock solution, a template volume ≤10% of the total reaction volume should be used.
6. qPCR reactions should be run in duplicate or triplicate.
7. The required volume of each component should be calculated based on the intended number of reaction setups, and an additional 10% volume of each component should be added to compensate for pipetting errors.
8. All common reaction components (primers and probes) should be added to the master mix and mixed thoroughly.
9. Appropriate volume of the reaction mix should be dispensed into the qPCR plates, and the plates should be sealed carefully with an optical sealing film.
10. Templates or NTC should be added to the wells containing the qPCR reaction mix.
11. The qPCR plates (tubes) should be centrifuged at 2500 rpm to sediment all contents at the bottom of the wells and obtain ready-to-use samples for qPCR reactions.

qPCR experiment setup:

A. Prepare the reaction mix.

1. Thaw TOOLS Easy 2×Probe qPCR Mix at room temperature and mix well to ensure no bubble formation. Spin down briefly in a microcentrifuge to sediment all contents to the bottom of tubes.

Component	Volume	Final concentration
TOOLS Easy 2×Probe qPCR Mix	10 μL	
Forward primer (10 μM)	0.4 μL	0.2 μM
Reverse primer (10 μM)	0.4 μL	0.2 μM
TaqMan probe (10μM)	0.2 μL	0.1 μM
cDNA or DNA template	X μL	
RNase-free ddH_2O	Up to 20 μL	

a. If the template is a stock solution, a template volume ≤1/10 of total reaction volume should be added.

B. qPCR program setup

Step	Temperature	Time	Cycle
Pollutant digestion	37 °C	2 min	1x
Initial denaturation	95 °C	5 min	1×
Denaturation	95 °C	10 s	45×
Annealing and extension	60 °C [*1]	30 s	

a. The polymerase included in this mix is a hot-start Taq DNA polymerase, and thus, the predenaturation stage should be set at 95°C for at least 5 minutes; however, it can be extended to 10 minutes if templates contain high GC content.

b. Extension time should be adjusted according to the qPCR instrument. For example, the extension time should be set to no less than 30 seconds when using ABI 7700 and 7900HT, 31 seconds when using ABI 7000 and 7300, 34 seconds when using ABI 7500, and 10 seconds when using ABI StepOnePlusTM.

The product has been developed only for research purpose and not for diagnostic and clinical use.