TOOLS One-Step Probe RT-qPCR Mix

For real-time RT-qPCR using probes

Contents

Introduction

TOOLS One-Step Probe RT-qPCR Mix is specially designed for rapid real-time PCR with probes (e.g., TaqMan®, Molecular Beacon). The kit allows both reverse transcription and gene amplification in a single tube, thus preventing cross-contamination and improving detection sensitivity.

25×TOOLS Enzyme Mix contains a highly efficient RTase expressed by engineering bacteria. This modified hot-start Taq DNA polymerase provides high efficiency and accuracy for amplification reactions and RNase inhibition. A specially modified hydrophobic motif enables this RTase to exhibit a considerable affinity for RNA and facilitates transcription of RNA templates, especially of those with high guanine–cytosine content or complex secondary structures. This product contains appropriate ion concentrations, dNTPs, and PCR enhancers. 25×TOOLS Enzyme Mix stabilizes polymerases and maintains their efficiency throughout the reaction process.

Important notes

1. The RNA template should be total RNA or mRNA.
2. To avoid RNase contamination, the operator must: i) wear disposable gloves and a breathing mask; ii) use RNase-free reagents, pipette tips, microtubes, and other instruments; and iii) perform experiments in areas dedicated to RNA operation.
3. 25×TOOLS Enzyme Mix should be centrifuged transiently before use and slowly pipetted; it should be stored at −20 °C soon after use.
4. The PreMix or Enzyme Mix should be completely mixed, and the reagent should be centrifuged to the bottom of the tube before use.
5. Specific reverse transcription primers should be used. Random Primers and Oligo dT Primers cannot be used in reverse transcription reactions.
6. To perform several one-step real-time RT-qPCRs simultaneously, all reagents should be combined and then divided evenly into each reaction tube. This limits reagent loss, avoids excessive addition of the same reagent, and minimizes error through adjustment of the volume of each component.

Kit can be used with the following devices:

1. PRISM 7000/7700/7900HT, 7300/7500 Real-Time PCR System, 7500 Fast Real-Time PCR System, ViiA 7 (Applied Biosystems)
2. OPTICONTM/CFX96 (BIORAD)
3. Light Cycler 480 (Roche)
4. Smart Cycler® System (Cepheid)
5. Mx3000P/Mx3005P (Stratagene)
6. Other real-time PCR thermal cyclers

Kit Contents

Contents	FPT-BC14 (50 mL × 50 rxn)
2×TOOLS PreMix	1.25 mL
25×TOOLS Enzyme Mix	100 μL
50×ROX Dye	250 μL
RNase-free ddH_2O	1 mL × 2

Storage

TOOLS One-Step Probe RT-qPCR Mix can be stored at −20 °C for up to 1 year.

Materials not supplied

1. Primers and probes
2. Templates

Protocol

1. Thaw RNA templates, primers, 2×TOOLS PreMix, 50×ROX Reference Dye, and RNase-Free ddH_2O. Centrifuge transiently and place on ice.
2. Prepare a reaction mixture according to the following table (all steps should be performed on ice).

Component	Volume	Final concentration
2×TOOLS PreMix	25 μL	-
25×TOOLS Enzyme Mix	2 μL	-
Forward primer (10 μM)	1.25 μL[*a]	0.25 μM
Reverse primer (10 μM)	1.25 μL[*a]	0.25 μM
Probe (10 μM)	1 μL[*b]	0.2 μM
RNA template	10 pg–1 μg total RNA	-
50×ROX Dye[*c]	-	-
RNase-free ddH_2O	To 50 μL	-

Notes:

a. A final primer concentration of 0.25 μM is optimal for most applications. However, for determination of optimal primer concentration in specific cases, primer titration from 0.05 to 0.9 μM can be performed. Increasing the concentration of the primer increases the amplification efficiency, and reducing the concentration of the primer reduces the nonspecific amplification.

b. The probe concentration differs with the RT-qPCR instrument, probe type, and fluorophore type. We recommend carefully reading the instructions of instruments and probes before use. A final probe concentration of 0.20 μM is optimal for most applications. However, for determination of optimal primer concentration in specific cases, probe titration from 0.1 to 0.5 μM can be performed.

c. The optimal concentrations of ROX Reference Dye for commonly used real-time PCR instruments are as follows:

Instrument	Final concentration
ABI PRISM 7000/7300/7700/7900HT/Step One	5× (e.g., 5 μL ROX/50 μL)
ABI 7500, 7500 Fast; Stratagene Mx3000P, Mx3005P, Mx4000, etc.	1× (e.g., 1 μL ROX/50 μL)
Roche, Bio-Rad, and Eppendorf instruments	Not required

3. One-Step Real-Time RT-qPCR

Centrifuge the PCR tubes, and place them into the fluorescence qPCR instrument for real-time PCR. The following table presents the recommended PCR program. PCR conditions should be further optimized if experimental results are not satisfactory.

Cycles	Temperature	Time	Step
1	50 °C	30 min	Reverse transcription
1	95 °C	3 min	Initial denaturation
40	95 °C	15 s	Denaturation
	60 °C [*a]	30 s	Annealing and extension. Collection of fluorescent signal

4. Result Analysis

After reaction, confirm the amplification curves and CT values, draw the standard curve, and analyze the results.

The product is for research only, not for diagnostic or clinical use.