

2×One-step Real-time PCR Premixture (Probe)

Cat. # 7601

Storage: stable up to one year at -20°C.

Description:

2×One-step Real-time qPCR Premixture (Probe) is designed to perform RT-qPCR in a single tube. As the mixture of all components necessary for RT-PCR is prepared in one tube, it is unnecessary to addition successively of reagents in the middle of the process, and a risk of contamination is minimized. This kit includes all reagents necessary for the reverse transcription of RNA to cDNA and cDNA amplification using PCR. These premixed components make it extremely easy to prepare a reaction mixture for quantification (Probe).

Component:

Component	PR7601 (50 reactions)	PR7602 (100 reactions)
RT-PCR MIX	125 µl	250 µl
2×One-step Probe Mix	1.25 ml	2.5 ml

Supplied by users:

RNase-free water

RNA template

Specific primers

Probe

Procedure:

1. Prepare the reaction mixture

Component	20µl	25µl	50µl	Final Conc.
2×One-step Probe Mix	10µl	12.5µl	25µl	1×
Upstream Primer				100-400nM
Downstream Primer				100-400nM
Template RNA				Pg-ng
Probe				50-300nM
RT-PCR MIX	1 µl	1.25 µl	2.5 µl	
Sterile water	Up to 20µl	Up to 25µl	Up to 50µl	

2. Mix gently and start RT-PCR under next program:

Two-step PCR

Cycle numbers	Step	Temperature	Time	Detection
1	1	50°C	20-30 min	off
1	1	95°C	5 min	off
35-45	1	95°C	15 s	off
	2	60-68°C	20-60 s	on

Three-Step PCR

Cycle numbers	Step	Temperature	Time	Detection
1	1	50°C	20-30 min	off
1	1	95°C	5 min	off
35-45	1	95°C	15 s	off
	2	55-65°C	10-20 s	off
	3	72°C	20-60 s	on

Note:

1. Oligo (dT) and random primers is not suitable for this kit.
2. Prepare reaction mixture on ice to avoid non-specific reaction.
3. In real-time qPCR, ROX often as the passive dye, provides an internal reference to normalize non-PCR related fluctuations in fluorescence (e.g. pipetting errors) and machine “noise”, also provides a stable baseline for qPCR. **Signalway Biotechnology real-time qPCR products are universal reagents that can be applied in any real-time thermal cyclers**, such as ABI PRISM7000/7700/7900HT, 7300/7500 Real-time PCR system, 7500 Fast Real-time PCR System (Applied Biosystems), ABI GeneAmp5700, StratageneMx3000, Mx3005P, Mx4000, and Bio-Rad instruments. ROX is not necessary for experiments if application plots and dissociation curve are ideal after optimize reaction system and thermal program.