Tinzyme Co., Limited

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One Step RT-qPCR Probe Kit

Product Number: PCK07

Shipping and Storage

-20°C in dark; If used frequently, store at 2-8°C, avoiding repeated freezing and thawing.

Components

Component	PCK07 100rxns	PCK07L 100rxns	PCK07H 100rxns
2× One Step Buffer	1.4 ml	1.4 ml	1.4 ml
One Step EnzymeMix	100 µl	100 µl	100 µl
50×Low ROX	-	50 µl	-
50×High ROX	-	-	50 µl
RNase-Free Water	1.5 ml	1.5 ml	1.5 ml

Description

This product is a specialized reagent kit for one-step Real Time RT qPCR using probe methods (TaqMan, Molecular Beacon, etc.).When using this product for Real Time RT-qPCR reaction, reverse transcription and quantitative PCR are carried out in the same reaction system, and there is no need to add reagents or open the tube cap during the reaction process, which avoids pollution and improves experimental efficiency.This product has high detection sensitivity, strong fluorescence signal, and high signal-to-noise ratio, making it very suitable for the detection of trace amounts of RNA such as RNA viruses. The special buffer system contained in it can maximize the effectiveness of both reverse transcriptase and DNA polymerase, improving reaction efficiency.Using this product can obtain a wider linear range, more accurate quantification of the target gene, good repeatability, and high reliability.

ROX dye is used to correct the fluorescence signal error generated between wells in quantitative PCR instruments, and is generally used in Real Time PCR amplification instruments from companies such as ABI and Stratagene.The excitation optical systems of different instruments vary, so the concentration of ROX dye must be matched with the corresponding fluorescence quantitative PCR instrument.

Instruments without ROX correction:Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, etc.

Instruments requiring Low ROX correction:ABI Prism7500/7500 Fast, QuantStudio® 3 System, QuantStudio® 5 System, QuantStudio® 6 Flex System, QuantStudio® 7 Flex System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000, etc.

Instruments requiring High ROX calibration:ABI Prism7000/7300/770/7900, Eppendorf, ABI Step One/Step One Plus, etc.

Notes

1. Please mix all the reagents in this kit gently upside down before use, trying to avoid foaming; then centrifuge briefly.
2. This product uses RNA as a template for one-step RT-PCR experiments and should avoid RNase contamination during the operation. It is recommended to perform RNA operations in a dedicated area, using specialized instruments and consumables. operators should wear masks and one-time use gloves and change gloves frequently. Related consumables are treated with 0.1% DEPC (diethylpyrocarbonate) at 37°C for 12 hours and are autoclaved for 30 minutes.
3. The reagents in this kit should avoid repeated freezing and thawing. Repeated freezing and thawing may degrade product performance.
4. The kit must use specific primers. Primers can be selected according to specific experiments. Primers design will directly affect the result of RT-PCR reactions. When designing primers, many factors need to be considered, such as GC content, primer length, primer position, secondary structure of PCR products, etc. It is recommended to use professional software to design.
5. This kit recommends the use of specific probes. It is recommended to use professional software for design.

Protocol:

The following is an example of conventional reaction system and reaction condition. The actual protocol should be based on the template, the structure of the primer and the size of the target fragment to make corresponding improvements and optimization(the preparation of reaction solutions should be on ice).

1. The RNA template, primers,2×One Step Buffer, One Step Enzyme Mix, and RNase-Free Water are thawed and placed on ice for use.
2. PCR reaction system:

Reagent	25μL reaction system	Final Concentration
2×One Step Buffer	12.5μl	1×
Forward Primer, 10μM	0.5μl	0.2μM [1)]
Reverse Primer, 10μM	0.5μl	0.2μM [1)]
Probe, 10μM	0.5μl	0.2μM [2)]
One Step Enzyme Mix	1.0μl	
RNA Template	Xμl	10 pg – 100 ng[3)]
50×Low ROX or High ROX(optional)[4)]	0.5μl	1×
RNase-Free Water	up to 25μl	

Note:1) Generally, a good result can be obtained with a primer concentration of 0.2μM, and a final concentration should be between 0.1-1.0μM.

2) The concentration of the probe to be used depends on the type of qPCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or adjust the concentration according the specific application requirements of each probe.

3) Usually, the amount of RNA template is 10 pg-100ng as a reference. Because of the difference of the copy number of the target gene in the different species' templates, the templates can be diluted serially to determine the optimal template amount.

4) The excitation optics of the different instruments are different. 50×Low ROX or 50×High ROX is selected according to the qPCR machine used.

3. Vortex to mix, briefly centrifuge, and collect the solution at the bottom of the tube.
4. RT-qPCR reaction condition:

Step	Temperature	Time	
Reverse transcription	45℃	10min	
Pre-denaturation	95℃	10min[1)]	
Denaturation	95℃	15 s	30-40cycles
Annealing/Extension[2)]	60℃	45s	

Note:1) The hot-start enzyme used in this product must be predenatured at 95°C for 10 minutes to activate the enzyme.

2)It is recommended to use two-step PCR. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR program, and set the annealing temperature between 56-64°C.