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SuperStar TaqMan One Step RT-qPCR Kit

Product Number: PCK95

Shipping and Storage

Stored at -20℃.If frequent use is required, it can be stored at 2-8 ℃ to avoid repeated freeze-thaw cycles as much as possible.

Components

Component	PCK95 100rxns
2×SuperStar TaqMan One Step Buffer	1.4ml
SuperStar TaqMan One Step Enzyme	100μl
RNase-Free Water	1ml

Description

This product is a specialized reagent kit for one-step Real Time RTqPCR 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, without the need to add reagents or open the tube cap during the reaction process, which avoids contamination and improves experimental efficiency.The transcription activity of the new reverse transcriptase in the reagent kit has been significantly improved. The new fast hot start enzyme has a fast start-up speed, high amplification efficiency, and good specificity. The special buffer system it contains can enable the reverse transcriptase and DNA polymerase to play their maximum role simultaneously, improving reaction efficiency.This product has high detection sensitivity, strong fluorescence signal and high signal-to-noise ratio, and is very suitable for the detection of RNA viruses and other microRNAs, such as the detection of novel coronavirus (2019-nCoV). Using this product can obtain a wider linear range, more accurate quantification of target genes, good repeatability, and high reliability.

Note

1. Before use, please gently mix the reagents in this reagent kit upside down to avoid foaming. After a brief centrifugation, use.
2. This product uses RNA as a template for one-step RT-PCR experiments. During the operation, RNase contamination should be avoided. It is recommended to perform RNA operations in a dedicated area using specialized instruments and consumables. Operators should wear masks and disposable gloves and frequently change gloves. The experimental consumables should be treated with 0.1% DEPC (diethyl pyrocarbonate) aqueous solution at 37 ℃ for 12 hours and sterilized under high pressure for 30 minutes before use.
3. Each reagent in this kit should avoid repeated freeze-thaw cycles as repeated freeze-thaw cycles may lead to a decrease in product performance.
4. This reagent kit must use specific primers, and the selection of primers can be based on specific experiments. The quality of primer design directly affects the results of RT qPCR reaction. When designing primers, factors such as GC content, primer length, primer position, and the secondary structure of PCR products need to be considered. It is recommended to use professional primer design software for design.
5. This reagent kit recommends the use of specific probes and professional design software for design.

Protocol

The following examples are typical reaction systems and conditions. In practical operation, corresponding improvements and optimizations should be made based on the differences in template, primer structure, and target fragment size. (Please prepare the reaction solution on ice)

1. Dissolve the RNA template, primers, 2×SuperStar TaqMan One Step Buffer、 SuperStar TaqMan One Step Enzyme and

RNase-Free Water,and place them on ice for later use.

2. PCR reaction system:

Reagent	20μL	Final Conc.
2×SuperStar TaqMan 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)]
SuperStar TaqMan One Step Enzyme	1.0μl	
RNA Template	Xμl	10 pg – 100 ng[3)]
RNase-Free Water	up to 25μl	

Note:1)Usually, a primer concentration of 0.2μM can yield good results, and can be used as a reference for setting the range from 0.1 to 1.0μM.

2)The concentration of the probe used is related to the fluorescent quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

3)Usually, the amount of RNA templates is based on 10 pg-100ng. Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be applied to the templates to determine the optimal template usage.

3. Mix well, centrifuge briefly, and collect the solution to the bottom of the tube.

4. RT-PCR reaction conditions:

Step	Temperature	Time	cycles
Reverse Transcription	50℃	15 min	
PCR pre denaturation	94℃	1 min [1)]	
Denaturation	94℃	15 s	40-45
Annealing/Extension [2)]	58℃	30 s	

Note:1)The hot start enzyme used in this product must be activated under pre denaturation conditions of 94℃ and 1 minute.

2)It is recommended to use a two-step PCR reaction program. If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification can be attempted. The annealing temperature should be set within the range of 56℃ -64℃ as a reference.