Tinzyme Co., Limited

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Multiplex One Step RT-qPCR, UNG Kit

Product Number: PCK49

Shipping and Storage

Stored at -30~-15°C, valid for 1 year. If frequent use is required, it can be packaged and stored at 2-8°C to avoid repeated freeze-thaw as much as possible

Components

Component	100rxns
4×Multiplex One Step RT-qPCR, UNG Kit Buffer	500 μL
Multiplex One Step RT-qPCR, UNG Enzyme	200 μL
RNase-Free Water	2×1 mL

Product Introduction

This product is a specialized reagent kit for one-step RT-qPCR using probe methods (TaqMan, Molecular Beacon, etc.).When using this product for 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. This kit introduces a dUTP-UNG anti pollution system, which can effectively remove residual contamination of PCR products and greatly reduce false positives caused by amplification product contamination.UNG enzyme can rapidly degrade pollutants containing U at room temperature without affecting the formation of new dU based PCR products.

Notes

1. Before using the reagents in this kit, please gently mix them upside down to avoid foaming, and use them after brief centrifugation.
2. This product uses RNA as a template for one-step RT-qPCR experiments. During the operation, RNase contamination should be avoided. It is recommended to perform RNA operations in a specialized 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°C for 12 hours and sterilized under high pressure for 30 minutes before use.
3. The reagents in this kit should avoid repeated freezing and thawing; This product can be stored at -20°C for long-term storage. If frequent use is required in the short term, it can be stored at 2-8°C.
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. It is recommended to use specific probes in this kit and use professional design software for design

Protocol

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

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1. Dissolve the RNA template, primer, 4×Multiplex One Step RT-qPCR, UNG Kit Buffer、Multiplex One Step RT-qPCR, UNG Enzymeand RNase Free Water and place them on ice for later use.
2. RT-qPCR reaction system:

Component	20μL reaction system	Final Concentration
4×Multiplex One Step RT-qPCR, UNG Kit Buffer	5 μL	1×
Multiplex One Step RT-qPCR, UNG Enzyme	2 μL	1×
Forward Primer	X μL	0.2 μM1)
Reverse Primer	X μL	0.2 μM1)
Probe	X μL	0.1 μM2)
RNA Template	X μL	10pg-100 ng3)
RNase-Free Water	Up to 20 μL	

Note:1)Typically, the primer concentration is 0.2μM can achieve good results, ranging from 0.1 to 1.0μM as a reference for setting the range.

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 10pg-100ng. Due to different species of templates containing different copies of the target gene, gradient dilution can be performed on the template to determine the optimal template usage.

3. Mix well, centrifuge briefly, and collect the solution to the bottom of the tube.
4. RT-qPCR reaction conditions:

Step	Temperature	Time	cycles
Reverse transcription	55℃	5min4)	1
Pre denaturation	96℃	20sec5)	1
Denaturation	96℃	5sec	40-45
Annealing/Extension/Fluorescence Collection	58℃6)	30sec7)	

Note:4)The reverse transcription time can be set within the time range of 1 minute to 5 minutes.

5)The hot start enzyme used in this product must activate the enzyme at a pre denatured temperature of 96℃ for at least 20 seconds.

6)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.

7)Different qPCR detection instruments require different fluorescence signal acquisition times. Please set according to the shortest time limit.