Version: 02/2024

WinScript One Step RT-qPCR U+ Kit

Cat. No. : CW3346S (200 rxns)
CW3346M (1000 rxns)

Storage Condition: -30~-15 °C storage. For frequent use, please store the buffer at 2-8 °C. Try to avoid repeated freeze-thaw.

Components

Component	CW3346S 200 rxns	CW3346M 1000 rxns
5×WinScript One Step RT-qPCR U+ Buffer	1 mL	5 mL
WinScript One Step RT-qPCR U+ Enzyme	200 μL	1 mL

Product Introduction

This product is a dedicated kit for one-step Real-Time RT-qPCR using the probe method (TaqMan, MolecularBeacon, etc.). When using this product for Real-Time RT-qPCR reactions, reverse transcription and quantitative PCR are performed in the same reaction system, and there is no need to add reagents or open the cap during the reaction, which avoids contamination and improves experimental efficiency. The dUTP/UNG anti-contamination system is introduced into this kit, which rapidly degrades U-containing contaminants at room temperature. This product has high detection sensitivity, strong fluorescence signal and high signal-to-noise ratio, which is very suitable for the detection of trace RNA such as RNA viruses. It contains a special buffer system that maximizes the efficiency of reverse transcriptase and DNA polymerase. The use of this product can obtain a wider linear range, more accurate quantification of the target gene, good reproducibility, and high reliability.

Precautions

1. Before using each reagent in this kit, gently invert it upside down to mix thoroughly, avoiding bubble formation as much as possible, and use it after brief centrifugation.
2. This product uses RNA as template for one-step RT-PCR experiments, therefore RNase contamination should be avoided during operation. It is recommended to perform RNA operations in a special area and use special instruments and consumables. Operators should wear masks and disposable gloves and change gloves frequently. Experiment-related consumables should be treated with 0.1% DEPC (diethyl pyrocarbonate) aqueous solution at 37 °C for 12 hours, and autoclaved for 30 minutes before use.
3. The reagents in this kit should avoid repeated freeze-thaw, which may reduce the performance of the product.
4. This kit must use specific primers. Primers can be selected according to the specific experiment. The quality of primer design directly affects the results of RT-qPCR reaction. When designing primers, factors to consider include GC content, primer length, primer location, and secondary structure of PCR products. It is recommended to use professional primer design software.
5. Specific probes are recommended for this kit, and professional design software is recommended for the design.

Product Introduction

The following examples show the conventional reaction system and reaction conditions, which should be improved and optimized according to the different templates, primer structures, and target fragment sizes.

1. Thaw the RNA template, primers, 5×WinScript One Step RT-qPCR U+ Buffer, WinScript One Step RT-qPCR U+ Enzyme and RNase-Free Water and set aside on ice.

2. Prepare the reaction according to the table below with a total volume of 25 μL.

Reagent	25 μL of Reaction	Final Concentration
5×WinScript One Step RT-qPCR U+ Buffer	5 μL	1×
WinScript One Step RT-qPCR U+ Enzyme	1 μL	
Forward Primer，10 μM	0.5 μL	0.2 uM[1)]
Reverse Primer，10 μM	0.5 μL	0.2 uM[1)]
Probe	0.25 μL	0.1 uM[2)]
RNA Template	X μL	10 pg-100 ng[3)]
RNase-Free Water	to 25 μL	

Note:
1) Usually, the primer concentration of 0.2 μM can give good results, and the final concentration of 0.1-1.0 μM can be set as a reference for setting range.
2) The concentration of the probe used is related to the type of fluorescence PCR instrument, probe type, and fluorescent label substance used. Please refer to the instrument manual or the specific use requirements of each fluorescent probe for concentration adjustment
3) Generally, the amount of RNA template is 10 pg-100 ng as reference. Because template contains different copies of the target gene in different species, the template can be gradient diluted to determine the optimal amount.

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

Steps	Temperature	Time	Cycles
Reverse Transcription	55 °C	5 min	1
Pre-Denaturation	95 °C	30 s	1
Denaturation	95 °C	5 s	45
Annealing, Extension and Fluorescence Collecting	58 °C[1)]	30 s	

Note:
1) It is recommended to use two-step PCR reaction procedure. If good experimental results are not obtained due to the use of primers with low Tm values, three-step PCR amplification can be attempted, the annealing temperature can be set in the range of 56 °C-64 °C as reference.