Version: 02/2024

4. RT-qPCR reaction conditions

Conventional reaction condition

Steps	Temperature	Time	Cycles
Reverse Transcription	50 ℃	5 min	1
Pre-Denaturation	95 ℃	30 s	1
Denaturation	95 ℃	10 s	45
Annealing, Extension and Fluorescence Collecting	58 ℃[1)]	30 s	

Rapid reaction condition

Steps	Temperature	Time	Cycles
Reverse Transcription	50 ℃	5 min	1
Pre-Denaturation	95 ℃	20 s	1
Denaturation	95 ℃	2 s	45
Annealing, Extension and Fluorescence Collecting	58 ℃[1)]	15 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, and the reference range of 56-64 ℃ can be used as reference.

2) The annealing time in the rapid program can be set according to the minimum default time of the instrument used.

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

Lyophilized WinScript Universal One Step RT-qPCR Mixture

Cat. No. : CW3363S (48 rxns)
CW3363M (100 rxns)

Storage Condition: 4-30 ℃ storage. After redissolution, it can be stored at -20 ℃. Avoid repeated freeze-thaw.

Components

Component	CW3363S 48 rxns	CW3363M 100 rxns
Lyophilized WinScript Universal One Step RT-qPCR Mixture	6×8-strip tubes	1×penicillin bottle

Introduction

Lyophilized WinScript Universal One Step RT-qPCR Mixture is a specialized lyophilized reagent for quantitative PCR reactions using RNA as a template. It includes a novel Taq DNA polymerase modified with antibodies, highly efficient heat-stable reverse transcriptase, enhancers, and stabilizers. The buffer contains Mg^{2+} and dNTP. Additionally, it incorporates factors that effectively suppress nonspecific PCR amplification and enhance the efficiency of multiplex qPCR reactions. This reagent enables efficient primer amplification while facilitating multiplex fluorescent quantitative amplification reactions. It is user-friendly, requiring only the addition of primers and probes, and the nucleic acid samples can be directly used for amplification after extraction. The product is compatible with both single-plex and multiplex probe-based qPCR reaction systems, suitable for conventional and rapid detection.

Precautions

This product can be stored at 4-30 ℃ for long-term storage and -20 ℃ for longer periods of time. If it is not used up after redissolution, it can be stored at -20 ℃. Avoid repeated freeze-thaw.

Protocol

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. PCR reaction system for 8-strip tubes

Reagent	25 μL System	Final Concentration
Lyophilized WinScript Universal One Step RT-qPCR Mixture	1 well	1×
Primer Probe Mix[1)]	X μL	
Template RNA[2)]	X μL	
RNase-Free Water	To 25 μL	

2. PCR reaction system for penicillin bottle

   Add 1.3 mL of ddH_2O to the penicillin bottle to redissolve, vortex and mix for 10 s, and briefly centrifuge for later use.

Reagent	25 μL System	Final Concentration
Redissolved Lyophilized WinScript Universal One Step RT-qPCR Mixture	12.5 μL	1×
Primer Probe Mix[1)]	X μL	
Template RNA[2)]	X μL	
RNase-Free Water	To 25 μL	

Note:

1) Generally, a better result can be obtained with a primer concentration of 0.2 μM. 0.1-1.0 μM can be set as a reference. The concentration of the probe used is related to the type of real-time PCR instruments, probe types, and fluorescent label substances used. Please refer to the instrument manual or the specific use requirements of each fluorescent probe for concentration adjustment.

2) 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.