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SuperRT V One Step RT-PCR Kit

Cat. No. : CW3377S (100 rxns)

Storage Condition: -30~-15℃.

Components

Component	CW3377S 100 rxns
SuperRT V OneStep EnzymeMix	100 μL
2×SuperRT V OneStep Buffer	1.25 mL
RNase-Free Water	2×1 mL

Introduction

This kit is specially developed for one-step RT-PCR experiments. Reverse transcription and PCR are carried out in the same reaction system, and there is no need to add reagents or open the tube cover during the reaction process, which improves the detection sensitivity and experimental efficiency while avoiding contamination. The kit includes a new high-efficiency reverse transcriptase, a rapid hot-start DNA polymerase, reaction buffers for reverse transcription and PCR amplification, and other components necessary for your experiment. SuperRT V reverse transcriptase lacks RNase H activity, reducing RNA degradation in reverse transcription reactions. This reverse enzyme has a high reverse transcription efficiency and can perform a good reverse transcription reaction on a small amount of RNA template. The rapid hot-start DNA polymerase used in the PCR reaction has excellent performance of high amplification efficiency, strong specificity, and fast extension speed. The unique buffer system allows for maximum efficiency of reverse transcriptase and polymerase. The target product amplified using this kit has an "A" base attached to the 3′ end and can be used directly for T/A cloning.

Notes

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross contamination in the experiment. It is recommended that RNA manipulation be carried out in a special area, using special instruments and consumables, and operators wear masks and disposable gloves and change gloves frequently.
2. In the experiment, disposable plastic utensils should be used. If glassware is used, it should be treated with 0.1% DEPC (diethyl pyrocarbonate) aqueous at 37 ℃ for 12 hours, and used after 30 minutes of autoclaved at 120 ℃, or glassware should be used after 60 minutes of dry heat sterilization at 180 ℃. Sterile water used in the experiment should be autoclaved after 0.1% DEPC treatment.
3. This kit should be used with specific primers. The selection of primers can be selected according to the specific experiment, and the quality of primer design directly affects the results of RT-PCR reaction. When designing the primers, GC content, primer length, primer position, secondary structure of PCR products and other factors should be considered. It is recommended to use professional primer design software.

4. All reagents in this kit should be gently mixed upside down before use, avoid foaming , and use after a short centrifugation. The enzymes involved should be put back to -20 ℃ as soon as possible after use to avoid repeated freeze-thaw.

Protocol

1. Thaw the 2×SuperRT V OneStep Buffer, SuperRT V OneStep EnzymeMix, primers, RNA template and RNase-Free Water, and place on ice for later use.
2. PCR reaction system:

Reagent	25 μL System	Final Concentration
2×SuperRT V OneStep Buffer	12.5 μL	1×
Forward Primer, 10μM	1 μL	0.4 μM
Reverse Primer, 10μM	1 μL	0.4 μM
SuperRT V OneStep EnzymeMix	1 μL	
RNA Template	X μL	1 pg – 1 μg
RNase-Free Water	to 25 μL	

Note:The final primer concentration of 0.1-0.5 μM can be set as a reference for setting range. The primer concentration can be increased when the amplification efficiency is not high, and the primer concentration can be reduced in the case of non-specific reactions, so that the reaction system can be optimized.

3. Vortex to mix, briefly centrifuge to collect the solution to the bottom of the tube.
4. RT-PCR reaction program:

Procedure	Temperature	Time	
Reverse Transcription	50 ℃	30 min	
Pre-denaturation	95 ℃	2 min	
Denaturation	95 ℃	30 sec	30-40 cycles
Annealing	55-65 ℃	30 sec	
Extension	72 ℃	30 sec	
Terminal Extension	72 ℃	5 min	

Note:1) In general experiments, the annealing temperature is 5 ℃ lower than the melting temperature (Tm) of the amplification primer, and the annealing temperature should be appropriately reduced when the ideal amplification efficiency cannot be obtained. When nonspecific reactions occur, increase the annealing temperature to optimize reaction conditions.
2) The extension time is set according to the size of the amplified fragment, and the amplification rate of DNA Polymerase included in this product is 1 kb/30s.
3) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too low, there is insufficient amplification. If the number of cycles is too high, the mismatch rate increases, leading to significant nonspecific background. Therefore, the number of cycles should be minimized on the premise of ensuring the yield of the product.

5. After the reaction, take 5 μL of the reaction product, and add an appropriate amount of loading buffer for electrophoresis to detect the results.