2) The extension time is set according to the size of the amplified fragment. The amplification efficiency of the DNA Polymerase included in this product is 1 kb/30s.
3) The cycle number can be set according to the downstream application of the amplified product. If the number of cycles is too small and the increment of expansion is insufficient. With more cycles, the mismatch rate will increase and the non-specific background will be serious. Therefore, under the premise of ensuring the yield of products, the number of cycles should be reduced as far as possible.

5. At the end of the reaction, 5 μL of reaction product was taken and appropriate amount of loading buffer was added for electrophoresis detection.

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

Version: 04/2023

SuperRT One Step RT-PCR Kit

Cat. No. : CW0742S (100 rxns)

Storage Conditions: -20°C.

Components

Component	CW0742S 100 rxns
SuperRT OneStep EnzymeMix	50 μL
2×SuperRT OneStep Buffer	1.4 mL
RNase-Free Water	1.5 mL

Principle

This kit is specially developed for one-step RT-PCR experiments. Reverse transcription and PCR are carried out in the same reaction system. 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. This kit includes a new high-efficiency reverse transcriptase, a rapid hot start DNA polymerase, and a reaction buffer for reverse recording and PCR amplification, as well as other components necessary for assays. SuperRT reverse transcriptase RNase H is inactive, which reduces the degradation of RNA in the reverse transcriptase reaction. The reverse enzyme has high reverse transcriptional efficiency and can perform a good reverse transcriptional response to a small amount of RNA template. The rapid hot start DNA polymerase used in PCR reaction has the advantages of high amplification efficiency, strong specificity and fast elongation. The unique buffering system maximizes both reverse transcriptase and polymerase. PCR products can be used directly for T/A cloning because most PCR products obtained by amplification are attached to the "A" base at the 3' end.

Cautions

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross-contamination in experiments. It is recommended that RNA manipulation be carried out in special areas, using special instruments and consumables, and that operators wear masks and disposable gloves and change gloves frequently.
2. Disposable plastic utensils should be used as far as possible in the experiment. If using glassware, 0.1% DEPC (diethyl pyrocarbonate) aqueous solution should be treated at 37°C for 12 hours and autoclaved at 120°C for 30 minutes before use, or the glassware should be sterilized at 180°C for 60 minutes after dry heat. The sterile water used in the experiment should be autoclaved with 0.1% DEPC.
3. Please mix all reagents in this kit upside down gently before use, avoid foaming as far as possible, and use after a short centrifugation. The enzymes involved should be returned to -20°C as soon as possible after use to avoid repeated freeze-thaw.
4. Specific primers must be used in this kit, and the selection of primers can be selected according to specific experiments. The design of primers directly affects the results of RT-PCR reaction. When designing primers, GC content, primer length, primer location, secondary structure of PCR products and other factors should be considered.

Procedure

1. The RNA template, primer, OneStep RT-PCR Buffer, SuperRT OneStep RT-PCR EnzymeMix and RNase-Free Water were dissolved and placed on ice for reserve.
2. The reaction system is formulated according to the following table:

Reagent	25 µL Reaction system	Final conc.
2×SuperRT OneStep Buffer	12.5 µL	
1× Forward Primer，10 µM	1 µL	0.4 µM
Reverse Primer，10 µM	1 µL	0.4 µM
SuperRT OneStep EnzymeMix	0.5 µL	
RNA Template	X µL	1 pg – 1 µg
RNase-Free Water	up to 25 µL	

Note: Primer concentration please use final concentration 0.1-1.0 µM as reference for the set range. When the amplification efficiency is not high, the concentration of primers can be increased. When non-specific reaction occurs, the concentration of primers can be reduced to optimize the reaction system.

3. The solution is collected to the bottom of the tube by vortex mixing and temporary centrifugation.
4. The thermal cycling apparatus was preheated to 45°C, and the PCR tube is placed in the thermal cycling apparatus for RT-PCR reaction.

Reaction conditions:

Step	Temperature	Time	
Reverse transcription	45°C	30 min	
PCR predenaturation	95°C	2 min	
denaturation	94°C	30 s	30-40 cycles
Annealing	55-65°C	30 s	
Extension	72°C	30 s	
Final extention	72°C	5 min	

Note:

1) In general PCR experiments, the annealing temperature is 5°C lower than the melting temperature Tm of amplification primers, and the annealing time is generally 20-30 seconds. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be appropriately reduced; In case of non-specific reaction, the annealing temperature is increased to optimize the reaction conditions.