SuperRT One Step RT-PCR Kit

Item No. S665660 (100 rxns)
Storage condition: -20℃

Product content

individual parts making up a compound	S665660 100 rxns
SuperRT OneStep EnzymeMix	50 μl
2×SuperRT OneStep Buffer	1.4 ml
RNase-Free Water	1.5 ml

Product Introduction

This kit is specially designed for one-step RT-PCR experiments. Reverse transcription and PCR are performed in the same reaction system, and there is no need to add reagents or open the cap of the tube, which avoids contamination and improves the sensitivity and efficiency of the experiments. The kit includes a new high-efficiency reverse transcriptase, fast hot-start DNA polymerase, reaction buffer for reverse transcription and PCR amplification, and other components necessary for the experiments.SuperRT Reverse Transcriptase RNase H is inactive, which reduces the degradation of RNA in the reverse transcription reaction. The reverse transcription efficiency of SuperRT reverse transcriptase is high, and it can perform good reverse transcription of small amount of RNA template.The fast hot-start DNA polymerase used in the PCR reaction has the excellent performance of high amplification efficiency, specificity, and fast extension speed. The unique buffer system allows the reverse transcriptase and polymerase to play their roles at the same time. The target product amplified by this kit has an ″A″ base at the 3′ end, which can be directly used for T/A cloning.

Caveat

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross-contamination in the experiment. It is recommended that RNA operation be

carried out in a special area, with special instruments and consumables, and that operators wear masks and disposable gloves and change gloves frequently.
2. Use disposable plastic containers as much as possible, and if glassware is used, it should be treated with 0.1% DEPC (diethylhexyl ether pyrocarbonate) aqueous solution at 37℃ for 12 hours and autoclaved at 120℃ for 30 minutes before use, or the glassware should be sterilized by dry heat at 180℃ for 60 minutes before use. Sterile water used in experiments should be treated with 0.1% DEPC and autoclaved.
3. All reagents in this kit should be mixed gently, upside down, to avoid foaming and centrifuged briefly before use. The enzymes should be returned to -20℃ as soon as possible after use to avoid repeated freezing and thawing.
4. This kit must use specific primers, primer selection can be selected according to specific experiments, primer design is good or bad directly affects the results of RT-PCR reaction, the design of primers need to consider the GC content, primer length, primer position, the secondary structure of the PCR product and other factors, it is recommended to use professional primer design software to design.

Usage

1. Dissolve RNA template, primers, OneStep RT-PCR Buffer, SuperRT OneStep RT-PCR EnzymeMix and RNase-Free Water and set aside on ice.
2. Prepare the reaction system according to the table below:

reagents	25 μl reaction system	final concentration
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: Please use the final concentration of 0.1-1.0 μM as a reference for setting the range of primer concentration. If the amplification efficiency is not high, the primer concentration can be increased; if a non-specific reaction occurs, the primer concentration can be decreased to optimize the reaction system.
3. Vortex and shake to mix, centrifuge briefly, and collect the solution at the bottom of the tube.

4. Preheat the thermocycler to 45° C, place the PCR tube in the thermocycler and perform the RT-PCR reaction.

Reaction conditions:

move	temp	timing	/
reverse transcription	45° C	30 min	/
PCR pre-denaturation	95° C	2 min	
denaturation	94° C	30 s	30-40 cycles
annealing (metallurgy)	55-65° C	30 s	30-40 cycles
reach	72° C	30 s	30-40 cycles
At last, an extension.	72° C	5 min	/

Attention:

(1) In general, the annealing temperature in PCR experiments is 5℃ lower than the melting temperature of the amplification primer Tm, and the annealing time is generally 20-30 seconds. When the desired amplification efficiency cannot be obtained, the annealing temperature should be lowered appropriately; when a non-specific reaction occurs, the annealing temperature should be raised, thus optimizing the reaction conditions.

2) The extension time is set according to the size of the amplified fragments, and the amplification efficiency of the 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 amplified product. If the number of cycles is too low, the amplification amount will be insufficient; if the number of cycles is too high, the chance of mismatch will increase and the non-specific background will be serious. Therefore, the number of cycles should be minimized under the premise of ensuring the product yield.

5. At the end of the reaction, 5 μl of the reaction product was taken and added to the appropriate amount of sampling buffer for electrophoretic detection of the results.