SuperRT cDNA Synthesis Kit

Item No. S665657 (100 rxns)
Storage condition: -20℃

Product content

individual parts making up a compound	100 rxns
SuperRT, 200 U/μl	100 μl
5×SuperRT Buffer	500 μl
Primer Mix	240 μl
dNTP Mix, 2.5 mM Each	500 μl
ddH2O	1 ml

Product Introduction

This is a cDNA first-strand synthesis kit designed for the first step of two-step RT-PCR. The reverse transcriptase used in this kit is a new high-efficiency reverse transcriptase for recombination and expression using E. coli engineered bacteria. RNase H activity has been removed and its thermal stability has been enhanced, allowing for the synthesis of the first strand of cDNA from very low amounts of total RNA or mRNA, with starting sample sizes as low as pg. The SuperRT Reverse Transcriptase has a high affinity for RNA, and is able to pass through RNA templates with high GC content and complex secondary structures to obtain high yields of cDNA. SuperRT Reverse Transcriptase has a high affinity for RNA and can read through RNA templates with high GC content and complex secondary structure to obtain high yields of cDNA. This product contains all the reagents needed to reverse transcribe RNA templates into the first strand of cDNA, including Super RT High Efficiency Reverse Transcriptase, Reaction Buffer, Primer, dNTP, etc., which is simple and convenient to use. The system is highly compatible with subsequent PCR and quantitative PCR experiments, and is suitable for a variety of PCR reactions with DNA polymerase.

Product Features

-Efficient reverse transcription: high affinity for RNA templates, reverse transcription efficiency up to 90%, recognizes pg level templates.
Freedom to cope with complex templates: even with high GC content and complex secondary structures, good results can be obtained without high-temperature denaturation.

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 for the experiment. If glassware is used, it should be treated with 0.1% DEPC (diethyl 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. If the amount of starting RNA is less than 50 ng, it is recommended to add RNAase inhibitor (RNasin). This kit is not supplied.

Usage

Note: 1 ng-5 μg of total RNA creates a 20 μl reaction system; if the amount of total RNA is greater than 5 μg, scale up the reaction system proportionally.
i Reverse transcription procedure:
1. Dissolve RNA template, Primer Mix, dNTP Mix, SuperRT Buffer, SuperRT and RNase-Free Water and set aside on ice.
2. Prepare the reaction system according to the following table in a total volume of 20 μl.

reagents	20 μl reaction system	final concentration
dNTP Mix, 2.5 mM Each	4 μl	500 μM Each

Primer Mix	2 μl	/
RNA Template	X μl	50 pg-5 μg
SuperRT, 200 U/μl	1 μl	/
RNase-Free Water	up to 20 μl	/

Attention:

1) If the amount of starting RNA is less than 50 ng, it is recommended to add RNAase inhibitor (RNasin). It is not provided in this kit.

(2) Primer Mix is made of Oligo(dT) and Random Primer. According to the needs of the experiment, Oligo-dT Primer or Gene Specific Primer can be used. It is recommended to use 50 pmol of Oligo-dT Primer or 2 pmol of Gene Specific Primer for 20 μl reaction system.

3. Mix by vortex shaking and centrifuge briefly so that the solution on the walls of the tube collects at the bottom.

4. Incubate at 42° C for 30-50 minutes and at 85° C for 5 minutes. At the end of the reaction, centrifuge briefly and place on ice to cool.

5. Reverse transcription products can be used directly in PCR and fluorescence quantitative PCR reactions or stored at -20° C for long term storage. Reagents 20 μl reaction system Final Concentration dNTP Mix, 2.5 mM Each 4 μl 500 μM Each Primer Mix 2 μl RNA Template X μl 50 pg-5 μg 5×SuperRT Buffer 4 μl 1× SuperRT, 200 U/μl 1 μl RNase-Free Water up to 20 μl

ii The following steps are recommended if reverse transcription efficiency is low, or if the RNA template secondary structure is complex and GC content is high:

1. Dissolve RNA template, Primer Mix, dNTP Mix, SuperRT Buffer, SuperRT and RNase-Free Water and set aside on ice.

2. Configure the reaction system according to the following table in a total volume of 15 μl.

reagents	20 μl reaction system	final concentration
dNTP Mix, 2.5 mM Each	4 μl	500 μM Each
Primer Mix	2 μl	/
RNA Template	X μl	50 pg-5 μg
RNase-Free Water	up to 15 μl	/

Note: The Primer Mix consists of Oligo(dT) and Random Primer. The Oligo-dT Primer or Gene Specific Primer can be used according to the needs of the experiment.

3. Incubate at 70° C for 10 minutes and rapidly ice bath for 2 minutes.

4. Centrifuge briefly so that the solution on the walls of the tube collects at the bottom.

5. Continue to add the following reagents to the above reaction solution:

reagents	20 μl reaction system	final concentration
5×SuperRT Buffer	4 μl	1×
SuperRT, 200 U/μl	1 μl	/

Note: If the amount of starting RNA is less than 50 ng, it is recommended to add RNAase inhibitor (RNasin). This kit is not supplied.

6. Incubate at 42° C for 30-50 minutes and at 85° C for 5 minutes.

7. At the end of the reaction, centrifuge briefly and cool on ice.

8. Reverse transcription products can be used directly in PCR and fluorescence quantitative PCR reactions or stored at -20° C for long periods of time.