
HiFiScript cDNA Synthesis Kit

Project number: H665509

Storage condition: -20°C

Product content

Component	H665509-100T
HiFiScript, 200U/ μ l	100 μ l
5 $\times$ RT Buffer	500 μ l
Primer Mix	240 μ l
dNTP Mix, 2.5 mM Each	500 μ l
DTT, 0.1M	240 μ l
RNase-Free Water	1ml

Product Introduction

This product is a cDNA first-strand synthesis kit specially formulated for the first step of two-step RT-PCR. The HiFiScript Reverse Transcriptase used in this kit is a new high-efficiency reverse transcriptase derived from M-MLV, with novel mutation sites that dramatically improve the transcriptional activity of the enzyme, and the efficiency and yield of cDNA first-strand synthesis is higher, and the first strand of cDNA can be synthesized using pg-level total RNA or mRNA. The point mutation eliminates RNase H activity and improves the elongation performance of the enzyme, which effectively improves the affinity of the reverse transcriptase to the RNA template, and allows for the production of cDNAs up to 12 kb. The carefully optimized RT Buffer allows for a wider range of applications of the reverse transcriptase, and provides high compatibility for subsequent PCR and quantitative PCR experiments. The kit comes with all reverse transcription reagents and is easy to use. It is suitable for first-strand cDNA synthesis and subsequent RT-PCR, RT-qPCR, and full-length cDNA library construction.

Product Features

1. Highly efficient reverse transcription efficiency: novel mutation sites dramatically increase enzyme activity for higher yields of cDNA.
2. Good elongation: point mutation eliminates RNase H activity and improves the affinity of reverse transcriptase for RNA templates, resulting in cDNAs up to 12 kb long.
3. High sensitivity: cDNA first strand can be synthesized using pg-level total RNA or mRNA templates.
4. Easy to use: The Reverse Transcription Kit comes with all the reverse transcription reagents, so you can easily complete the reverse transcription by simply preparing the template.

matters needing attention

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross-contamination in the experiment. It is recommended that RNA operations be performed in a dedicated area, specialized instruments and consumables be used, and that operators wear masks and disposable gloves and change gloves frequently.
2. Disposable plasticware should be used as much as possible. If glassware is used, it should be treated with 0.1% DEPC (diethylpyrocarbonate) aqueous

solution at 37°C for 12 hours and autoclaved at 120°C for 30 minutes before use, or the glassware should be dry heat sterilized at 180°C for 60 minutes before use. Sterile water used in the experiment should be treated with 0.1% DEPC and then autoclaved.

3. All reagents in this kit should be mixed gently before use by turning up and down to avoid foaming as much as possible, and after brief centrifugation. The enzymes involved should be put back to -20°C as soon as possible after use to avoid repeated freezing and thawing.

If the amount of starting RNA is less than 50ng, it is recommended to add RNAase inhibitor (RNasin).

Usage

Note: 1ng-5 µg of total RNA can establish a 20 µl reaction system, if the amount of total RNA is greater than 5 µg, please expand the reaction system proportionally.

Reverse transcription procedure:

1. Dissolve RNA template, Primer Mix, dNTP Mix, DTT, RT Buffer, HiFiScript 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	
5×RT Buffer	4 µl	1×
DTT, 0.1M	2 µl	10mM
HiFiScript, 200U/ µl	1 µl	
RNase-Free Water	up to 20 µl	

Note: 1) If the amount of starting RNA is less than 50ng, it is recommended to add RNAase inhibitor (RNasin).

2) Primer Mix was prepared from Oligo (dT) and Random Primer.

3. Vortex and shake to mix and centrifuge briefly so that the solution on the walls of the tube collects at the bottom.

4. cDNA synthesis reaction conditions:

1) If fluorescent quantitative PCR assay is performed downstream, incubate at 42° C for 15 minutes and 85° C for 5 minutes.

2) If downstream for normal PCR assay, incubate at 42° C for 30-50 minutes and 85° C for 5 minutes.

Note: For templates with complex secondary structure or high GC content, the reverse transcription temperature can be increased to 50° C to enhance reverse transcription efficiency.

5. At the end of the reaction, centrifuge briefly and place on ice to cool.

6. The reverse transcription product can be directly used in PCR reaction and fluorescence quantitative PCR reaction, or placed in -20°C for long-term storage.

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, DTT, RT Buffer, HiFiScript and RNase-Free Water and set aside on ice.
2. Prepare the reaction system according to the following table in a total volume of 13 μ l.

reagents	20 μ l reaction system	final concentration
dNTP Mix, 2.5 mM Each Primer Mix	4 μ l	500 μ M Each
RNA Template	2 μ l X μ l	
RNase-Free Water	up to 13 μ l	50pg-5 μ g

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 μ l reaction system	final concentration
5 $\times$ RT Buffer	4 μ l	1 $\times$
DTT, 0.1M	2 μ l	10mM
HiFiScript (200U/ μ l)	1 μ l	

Note: 1) If the amount of starting RNA is less than 50ng, it is recommended to add RNAase inhibitor (RNasin).

2) Primer Mix was prepared from Oligo (dT) and Random primer.

6. Perform cDNA first-strand synthesis:

1) If fluorescent quantitative PCR assay is performed downstream, incubate at 50° C for 15 minutes and 85° C for 5 minutes.

2) If downstream for normal PCR assay, incubate at 50° C for 30-50 minutes and 85° C for 5 minutes.

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

8. The reverse transcription product can be used directly in PCR reaction and fluorescence quantitative PCR reaction, the amount added should be less than 1/10 of the PCR reaction system, or placed in -20°C for long-term storage.