HiFi II M-MLV(H-) Reverse Transcriptase

Project No. H665664 (10,000 U)
Storage condition: -20℃ storage.

Product content

individual parts making up a compound	H665664 10000 U
HiFi II M-MLV(H-) (200U /μL)	50 μL
5×SuperRT Buffer	1 mL

Product Introduction

HiFi II M-MLV(H-) is a mutated M-MLV gene recombinant and expressed in E. coli engineered bacteria as a reverse transcriptase that catalyzes complementary DNA polymerization reactions using RNA or DNA:RNA hybrid strands as templates. The mutated HiFi II M-MLV(H-) reverse transcriptase is deficient in RNase H activity, which reduces the degradation of RNA in the reverse transcription reaction and makes it easier to obtain full-length cDNAs. HiFi II M-MLV(H-) reverse transcriptase is able to synthesize the first strand of cDNAs at 55° C, providing higher specificity, stability, and the ability to synthesize cDNAs of up to 12 kb with high cDNA yields. High cDNA yield. It is suitable for first-strand cDNA synthesis, RT-PCR, RT-qPCR and full-length cDNA library construction.

Active Definition

The amount of enzyme required to catalyze the incorporation of 1 nmol of dTTP in 10 min at 37° C is defined as one unit of activity (U), using Poly (A) as a template and oligo (dT) as a primer.

Quality control

200 U of this enzyme and 1 μg of 16 S, 23 S rRNA were reacted at 37° C for 1 h. The electrophoretic bands of RNA did not change.

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: 10 ng-5 μg of total RNA creates a 20 μL reaction system, if the amount of total RNA is greater than 5 μg, please scale up the reaction system proportionally.
i Reverse transcription procedure:
1. Dissolve RNA template, primers, dNTP Mix, SuperRT Buffer, HiFi II M-MLV(H-) 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
Oligo-dT Primer, 100 μM or Random Primers , 50 μM or Specific Primer , 10 μM	1 μl	/
RNA Template	X μl	1 ng-5 μg
5×SuperRT Buffer	4 μl	1 ×

HiFi II M-MLV(H-) (200U /μL)	0.5-1 μL	/
RNase-Free Water	up to 20 μL	/

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

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 55° C for 1-30 minutes and at 85° C for 5 minutes. At the end of the reaction, centrifuge briefly and place on ice to cool.

5. The reverse transcription product can be used directly in PCR and fluorescence quantitative PCR reactions, or placed at -20℃ for long-term storage.

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, primers, dNTP Mix, SuperRT Buffer, HiFi II M-MLV(H-) and RNase-Free Water and set aside on ice.

2. Prepare 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
Oligo-dT Primer, 100 μM or Random Primers , 50 μM or Specific Primer , 10 μ M	1 μl	/
RNA Template	X μl	1 ng-5 μg
RNase-Free Water	up to 15 μL	/

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. Add 4 μL of 5×SuperRT Buffer to the above reaction solution.

Note: If the amount of starting RNA is less than 50 ng, it is recommended that an RNAase inhibitor (RNasin) be added. It is not supplied with this kit.

6. Blow gently to mix, if the reverse transcription primer is an Oligo-dT Primer or Specific Primer.

7. Incubate at 42° C for 2 minutes or 25° C for 10 minutes if the reverse transcription primers are Random Primers.

8. Add 1 μL of HiFi II M-MLV(H-) (200 U/μL) and mix by gentle pipetting. Incubate at 55° C for 50 minutes. Incubate at 85° C for 5 minutes. At the end of the reaction, centrifuge briefly and place on ice to cool.
9. Reverse transcription products can be used directly in PCR reactions and fluorescence quantitative PCR reactions, or placed at -20° C for long-term storage.