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HiFi V M-MLV Reverse Transcriptase (RNase H-)

Product Number: RT07

Shipping and Storage

Store at -30 ~ -15 ℃ and transport on dry ice.

Components

Component	RT07 10KU	RT07 200KU	RT07 2000KU
HiFi V M-MLV Reverse Transcriptase (RNase H-) （200U /μL)	50 μL	1 mL	10 mL
5×SuperRT Buffer	1 mL	10 mL	100 mL

Description

HiFi V M-MLV Reverse Transcriptase (RNase H -) is a reverse transcriptase that recombines and expresses mutated M-MLV genes in E. coli engineering bacteria. This enzyme can catalyze complementary DNA polymerization reactions using RNA or DNA: RNA hybrid chains as templates. The mutated HiFi V M-MLV Reverse Transcriptase (RNase H -) RNase H activity is missing, reducing RNA degradation in reverse transcription reactions and making it easier to obtain full-length cDNA. HiFi V M-MLV Reverse Transcriptase (RNase H -) exhibits excellent reverse transcription activity at 55 ℃ (the enzyme can be used for reverse transcription reaction at up to 60 ℃). For complex RNA structures, increasing the reverse transcription reaction temperature can significantly improve the yield of cDNA. In addition, the mutated HiFi V M-MLV Reverse Transcriptase (RNase H -) has better stability and can synthesize up to 15kb of cDNA. Suitable for the synthesis of first stranded cDNA, RT PCR, RT qPCR, and construction of full-length cDNA libraries.

Unit definition

Using Poly (A) as a template and oligo (dT) as a primer, the enzyme required to catalyze the addition of 1 nmol of dTTP within 10 minutes at 37 ℃ is defined as one active unit (U).

Quality control

200 U of this enzyme reacted with 1μg of 16S, 23S rRNA at 37 ℃ for 1 hour, and the electrophoresis band of the RNA remained unchanged.

Note

1. During the operation process, RNase contamination should be avoided to prevent RNA degradation or cross contamination during experiments. It is recommended to perform RNA operations in specialized areas, use specialized instruments and consumables, wear masks and disposable gloves, and frequently change gloves.
2. Try to use disposable plastic containers for experiments. If using glassware, a 0.1% DEPC (diethyl pyrocarbonate) aqueous solution should be treated at 37 ℃ for 12 hours and sterilized under high pressure at 120 ℃ for 30 minutes before use. Alternatively, dry heat sterilize the glassware at 180 ℃ for 60 minutes before use. The sterile water used in the experiment should be treated with 0.1% DEPC and then subjected to high-pressure sterilization.
3. All reagents in this reagent kit should be gently mixed upside down before use, avoiding foaming as much as possible, and used after brief centrifugation. The enzymes involved should be returned to an environment below -15 ℃ as soon as possible after use to avoid repeated freeze-thaw cycles.
4. If the initial amount of RNA is less than 50 ng, it is recommended to add RNA enzyme inhibitors (RNAsin). This reagent kit is not provided. If needed, you can order it separately from our company, item number: RNK3501.

Protocol

A total RNA of 10ng-5ug can establish a 20uL reaction system. If the total RNA amount is greater than 5ug, please expand the reaction system proportionally.

1. Reverse transcription steps:

1.1. Dissolve RNA templates, primers, dNTP Mix, SuperRT Buffer, HiFi V M-MLV Reverse Transcriptase (RNase H -), and RNase Free Water and place on ice for later use.

1.2. Prepare the reaction system according to the following table, with a total volume of 20uL.

Reagent	20μlReaction system	Final concentration
dNTP Mix，2.5 mM Each	4μL	500μM Each
Oligo-dT Primer,100μM or Random Primers,50 μM or Specific Primer,10μM	1μL	
RNA Template	XμL	1 ng-5 μg
5×SuperRT Buffer	4μL	1 ×
HiFi V M-MLV Reverse Transcriptase (RNase H-) (200U /μL)	0.5-1μL	
RNase-Free Water	up to 20μL	

Note: If the initial amount of RNA is less than 50ng, it is recommended to add RNA enzyme inhibitors (RNasins). This reagent kit is not provided. If needed, you can order it separately from our company, item number: RNK3501.

1.3. Vortex oscillate and mix well, briefly centrifuge to collect the solution on the tube wall to the bottom of the tube.

1.4. Incubate at 55 ℃ for 1-15 minutes, and incubate at 85 ℃ for 5 minutes. After the reaction is complete, centrifuge briefly and cool on ice.

1.5. Reverse transcripts can be directly used for PCR reactions and fluorescence quantitative PCR reactions, or stored for a long time at -20 ℃ or below.

2. If the reverse transcription efficiency is low, or the RNA template secondary structure is complex and the GC content is high, the following steps are recommended:

2.1. Dissolve RNA templates, primers, dNTP Mix, SuperRT Buffer, HiFi V M-MLV Reverse Transcriptase (RNase H -), and RNase Free Water and place on ice for later use.

2.2. Prepare the reaction system according to the following table, with a total volume of 15uL.

Reagent	20μlReaction system	Final concentration
dNTP Mix，2.5 mM Each	4μL	500μM Each
Oligo-dT Primer,100μM or Random Primers,50 μM or Specific Primer,10μM	1μL	
RNA Template	XμL	1 ng-5 μg
RNase-Free Water	up to 15μL	

2.3. Incubate at 70 ℃ for 10 minutes and quickly ice bath for 2 minutes.

2.4. Short centrifugation allows the solution on the tube wall to be collected to the bottom of the tube.

2.5. Add 4uL of 5 x SuperRT Buffer to the above reaction solution.

Note: If the initial amount of RNA is less than 50 ng, it is recommended to add RNA enzyme inhibitors (RNasins). This reagent kit is not provided and can be ordered separately from our company if needed,

2.6. Gently blow and mix well. If the reverse transcription primer is Oligo dT Primer or Specific Primer, incubate at 42 ℃ for 2 minutes; If the reverse transcription primer is Random Primers, incubate at 25 ℃ for 10 minutes.

2.7. Add 1uL HiFi V M-MLV Reverse Transcriptase (RNase H -) (200 U/uL), gently pipette and mix well. Incubate at 55 ℃ for 30 minutes.

2.8. Incubate at 2.8.85 ℃ for 5 minutes. After the reaction is complete, centrifuge briefly and cool on ice.

2.9. Reverse transcripts can be directly used for PCR reactions and fluorescence quantitative PCR reactions, or stored for a long time at -20 ℃ or below.