Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

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

Product Number: RT05

Shipping and Storage :

-20°C

Components

Component	RT05 10000U
HiFi II M-MLV(H-) (200U /μL)	50 μL
5×SuperRT Buffer	1 mL

Description

HiFi II M-MLV(H-) is a reverse transcription enzyme in which the mutant M-MLV gene is recombined and expressed by Escherichia coli engineering bacteria. The enzyme can catalyze the polymerization of complementary DNA using RNA or DNA: RNA hybrid chain as template. The mutation of HiFi II M-MLV reverse transcriptase RNase H activity is lost, reducing the degradation of RNA in reverse transcription and facilitating the acquisition of full-length cDNA. HiFi II M-MLV reverse transcriptase can synthesize the first strand cDNA at 55°C, providing higher specificity and stability, and can synthesize up to 12 KB cDNA with high cDNA yield. It is suitable for synthesis of first strand cDNA, RT-PCR, RT-QPCR and construction of full-length cDNA library.

Activity Definition

Using Poly (A) as template and Oligo (dT) as primer, the amount of enzyme required for catalytic incorporation of 1 nmol dTTP within 10 minutes was defined as an activity unit (U) at 37°C.

Quality Control

The electrophoretic bands of RNA did not change after the reaction of 200 U of this enzyme with 1 μg of 16 S and 23 S rRNA at 37°C for 1 hour.

Note

1. RNase contamination should be avoided during operation to prevent RNA degradation or crosscontamination in the experiment. It is recommended that RNA manipulation be carried out in a special area, using special instruments and consumables, and operators wear masks and disposable gloves and change gloves frequently.
2. In the experiment, disposable plastic utensils should be used. If glassware is used, 0.1% DEPC (diethyl pyrocarbonate) aqueous solution should be treated at 37°C for 12 hours, and used after 30 minutes of autoclaved at 120°C, or glassware should be used after 60 minutes of dry heat sterilization at 180°C. Sterile water used in the experiment should be autoclaved after 0.1% DEPC treatment.
3. Before use, please mix all reagents in this kit and gently to avoid foaming, and use after a short centrifugation. The enzymes involved should be put back to -20°C as soon as possible after use to avoid repeated freeze-thaw.
4. If the initial amount of RNA is less than 50 ng, RNA enzyme inhibitor (RNasin) is recommended. This kit is not provided, if necessary, you can order separately from our company.

Protocol

Note:20μL reaction system can be established by 10 ng-5μg total RNA, if the total RNA amount is more than 5μg, please scale up the reaction system.

1. Steps of reverse transcription:
   1.1. RNA templates, primers, dNTP Mix, SuperRT Buffer, HiFi II M-MLV(H-)and RNase-free Water were dissolved and placed on ice for later use.
   1.2. Prepare the reaction system according to the following table, the total volume is 20 μL.

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

Note: If the initial AMOUNT of RNA is less than 50 ng, RNA enzyme inhibitor (RNasin) is recommended. This kit is not provided, if necessary, you can order separately from our company.

   1.3. Vortex and mix thoroughly, temporary centrifugation, so that the solution on the wall of the tube to collect to the bottom of the tube.
   1.4. Incubate at 55°C for 1-15 minutes and at 85°C for 5 minutes. After the reaction, centrifuge briefly and cool on ice.
   1.5. The reverse transcription products can be directly used for PCR reaction and fluorescence quantitative PCR reaction, or stored at -20°C for a long time.
2. If the reverse transcription efficiency is low, or the secondary structure of RNA template is complex and GC content is high, the following steps are recommended:
   2.1. RNA templates, primers, dNTP Mix, SuperRT Buffer, HiFi II M-MLV(H-)and Rnase-free Water were dissolved and placed on ice for later use.
   2.2. Prepare the reaction system according to the following table, the total volume is 15 μL.

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

Note: If the initial AMOUNT of RNA is less than 50 ng, RNA enzyme inhibitor (RNasin) is recommended. This kit is not provided, if necessary, you can order separately from our company.

   2.3. Incubate at 70°C for 10 minutes and take a quick ice bath for 2 minutes.
   2.4. Centrifuge briefly to collect the solution from the wall to the bottom of the tube.
   2.5. Add 4μL 5×SuperRT Buffer to the above reaction solution.

Note: If the initial amunt of RNA is less than 50 ng, RNA enzyme inhibitor (RNasin) is recommended. This kit is not provided, if necessary, you can order separately from our company.

   2.6. When oligo-DT Primer or Specific Primer was used for reverse transcription, incubate for 2 minutes at 42°C. If the reverse transcription primer was Random Primers, it was incubated at 25°C for 10 minutes.
   2.7. Add 1μL HiFi II M-MLV(H-) (200U /μL), mix it gently with a pipette, incubate at 55°C for 50 minutes.
   2.8. Incubate at 85°C for 5 minutes. After the reaction, centrifuge briefly and cool on ice.
   2.9. The reverse transcription products can be directly used for PCR reaction and fluorescence quantitative PCR reaction, or stored at -20°C for a long time.