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

Product Number:RT05F

Shipping and Storage

Stored at -30~-15℃, Dry ice transportation to avoid repeated freezing and thawing.

Components

Component	RT05F 10000U	RT05F 200KU	RT05F 2000KU
HiFi II M-MLV Reverse Transcriptase (RNase H-) (Glycerol-free) (200U /μL)	50μL	1ml	10ml
5×SuperRT Buffer	1ml	10ml	100ml

Description

HiFi II M-MLV Reverse Transcriptase (RNase H-) (Glycerol-free) is the glycerol free version of HiFi II M-MLV Reverse Transcriptase (RNase H-),it is a reverse transcriptase that recombines and expresses the mutated M-MLV gene using Escherichia coli engineering bacteria,this enzyme can catalyze complementary DNA polymerization reactions using RNA or DNA: RNA hybrid chains as templates.The mutated HiFi II M-MLV (H -) reverse transcriptase RNase H activity is missing, reducing RNA degradation in reverse transcription reactions and making it easier to obtain full-length cDNA.HiFi II M-MLV (H -) reverse transcriptase can synthesize the first strand of cDNA at 55℃, providing higher specificity and stability. It can synthesize up to 12kb of cDNA with high cDNA yield.Suitable for the synthesis of first strand cDNA, RT-PCR, RT-qPCR, construction of full-length cDNA libraries, and application of freeze-dried RT-PCR products.This product does not contain freeze-dried shaping ingredients, and can be customized and added according to needs when applied to freeze-dried products.

Unit definition

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

Quality Control

The electrophoresis bands of the RNA did not change when 200U of this enzyme reacted with 1μg of 16S, 23S rRNA at 37 ℃ for 1 hour.

Notes

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 using specialized instruments and consumables. Operators should wear masks and disposable gloves and frequently change gloves.
2. Disposable plastic containers should be used as much as possible for experiments. If glass containers are used, they should be treated with a 0.1% DEPC (diethyl pyrocarbonate) aqueous solution at 37℃ for 12 hours and sterilized under high pressure at 120℃ for 30 minutes before use, or dry heat sterilized at 180℃ for 60 minutes before use. The sterile water used in the experiment should be treated with 0.1% DEPC and subjected to high-pressure sterilization.
3. Before use, all reagents in this reagent kit should be gently mixed upside down to avoid foaming and used after brief centrifugation. The enzymes involved should be returned to -20℃ as soon as possible after use to avoid repeated freezing and thawing.
4. If the initial amount of RNA is less than 50ng, it is recommended to add RNA enzyme inhibitors (RNase inhibitor, Murine).

Protocol

Note:10ng-5μg total RNA can establish 20μL reaction system, if the total RNA content is greater than 5μg. Please expand the reaction system proportionally.

1. Reverse transcription steps
   1.1. Dissolve the RNA template, primer, dNTP Mix,5×SuperRT Buffer,HiFi II M-MLV Reverse Transcriptase (RNase H-) (Glycerol-free),RNase Free Water and place them on ice for later use
   1.2. Prepare the reaction system according to the following table, with a total volume of 20μL.

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

Note: If the amount of initial RNA is less than 50ng, it is recommended to add RNA enzyme inhibitors (RNase inhibitor, Murine).

   1.3. Vortex oscillation and mixing, brief centrifugation to collect the solution on the tube wall to the bottom of the tube.
   1.4. Incubate at 55℃ for 1-30 minutes, and incubate at 85℃ for 5 minutes. After the reaction, centrifuge briefly and cool on ice.
   1.5. Reverse transcripts can be directly used for PCR reactions and fluorescence quantitative PCR reactions, or stored at -20℃ and used within six months; Long term storage is recommended to be stored at -80℃ after packaging. CDNA should avoid repeated freeze-thaw.
2. If the reverse transcription efficiency is low, or the secondary structure of the RNA template is complex and the GC content is high, the following steps are recommended:
   2.1. Dissolve the RNA template, primer, dNTP Mix,5×SuperRT Buffer,HiFi II M-MLV Reverse Transcriptase (RNase H-) (Glycerol-free),RNase Free Water and place them on ice for later use
   2.2. Prepare the reaction system according to the following table, with a total volume of 15μL.

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

3. Incubate at 70℃ for 10 minutes and quickly ice bath for 2 minutes.
4. Short centrifugation to collect the solution on the tube wall to the bottom of the tube.
5. Add 4μL of 5× SuperRT Buffer to the above reaction solution.

Note: If the amount of initial RNA is less than 50ng, it is recommended to add RNA enzyme inhibitors (RNase inhibitor, Murine).

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.
7. Join 1μL HiFi II M-MLV Reverse Transcriptase (RNase H -) (Glycerol free) (200U/μL) Gently suck and beat until well mixed. Incubate at 55℃ for 50 minutes.
8. Incubate at 85℃ for 5 minutes. After the reaction, centrifuge briefly and cool on ice.
9. Reverse transcripts can be directly used for PCR reactions and fluorescence quantitative PCR reactions, or stored at -20℃ and used within six months; Long term storage is recommended to be stored at -80℃ after packaging. CDNA should avoid repeated freeze-thaw.