Tinzyme Co., Limited

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

HiFi II MMLV Reverse Transcriptase A16

Product Number: IIA16

Storage condition

-20°C

Product component

HiFi II MMLV (RNase H-) (200U/uL)	customized
5×Super RT Buffer	customized

Description

HiFi II MMLV (H-) is a reverse transcriptase that uses engineered Escherichia coli to recombine and express mutated MMLV gene. This enzyme can catalyze complementary DNA polymerization using RNA or DNA: RNA hybrid chain as template. The mutated HiFi II MMLV (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 MMLV (H-) reverse transcriptase can synthesize the first strand of cDNA at 55 °C, providing higher specificity and stability. It can synthesize up to 12 kb of cDNA with high cDNA yield. Suitable for the synthesis of first strand cDNA, RT PCR, RT qPCR, and the construction of full-length cDNA libraries. The enzyme has been optimized with a unique A16 modification. IIA16 is a customized MMLV Reverse Transcriptase.

Unit definition

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

Quality control

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

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 a dedicated area, 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. Alternatively, glass containers should be sterilized under dry heat 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 50 ng, it is recommended to add RNA enzyme inhibitors.

Protocol

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

Reverse transcription steps:

1. Dissolve RNA templates, primers, dNTP Mix, Super RT Buffer, HiFi II MMLV (H-), and RNase Free Water and place them

on ice for later use.

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

Reagent	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×Super RT Buffer	4 μL	1 x
HiFi II MMLV(H-) (200U/μL)	0.5-1 μL	
RNase-Free Water	up to 20 μL	

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

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

4. Incubate at 55 °C for 1-30 minutes, and incubate at 85 °C for 5 minutes. After the reaction, centrifuge briefly and cool on ice.

5. Reverse transcripts can be directly used for PCR reactions and fluorescence quantitative PCR reactions, or stored at -20 °C for a long time.

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

1. Dissolve RNA templates, primers, dNTP Mix, Super RT Buffer, HiFi II MMLV (H-), and RNase Free Water and place them on ice for later use.

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

Reagent	15 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
RNase-Free Water	up to 15 μL	

3. Incubate at 70 °C for 10 minutes and quickly ice bath for 2 minutes.

4. Centrifuge briefly to collect the solution on the tube wall to the bottom of the tube.

5. Add 4 μL 5x Super RT Buffer to the above reaction solution.

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

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

7. Add 1 μL HiFi II MMLV(H-) (200 U/μL), Gently suck and beat until well mixed. Incubate at 55 °C for 50 minutes.

8. Incubate at 85 °C 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 °C for a long time.