

Catalog
TBP0154-5000

Unit
5000U

Description

HiFi II M-MLV (H-) is a reverse transcriptase enzyme derived from a mutant M-MLV gene, expressed in Escherichia coli engineering bacteria. It catalyzes the synthesis of complementary DNA (cDNA) using RNA or RNA-DNA hybrid templates. This mutant version lacks RNase H activity, which reduces RNA degradation during reverse transcription and enables the efficient synthesis of full-length cDNA. HiFi II M-MLV (H-) is ideal for first-strand cDNA synthesis, RT-PCR, RT-qPCR, and the construction of full-length cDNA libraries.

Product Details

Storage: -20°C

Components

Component
HiFi II M-MLV(H-) (200U / μ L)
5 \times Super RT Buffer

Protocol

Note: 20 μ L reaction system can be established by 10 ng-5 μ g total RNA, if the total RNA amount is more than 5 μ g, 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 μ L.

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

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 μ L

Reagent	20 μ L reaction system	Final concentration
dNTP Mix, 2.5 mM Each	4 μ L	500 μ M Each Oligo-dT
Oligo-dT Primer, 100 μ M or Random Primers, 50 μ M or Specific Primer, 10 μ M	1 μ L	
RNA Template	X μ L	1ng-5 μ g
RNase-Free Water	Up to 15 μ L	

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 4 μ L 5 \times SuperRT Buffer to the above reaction solution. **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.
- 2.6. 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 1 μ L HiFi II M-MLV(H-) (200U / μ L), 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.

For research use only