ii. 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:

1. Thaw RNA templates, primers, dNTP Mix, 5×SuperRT Buffer, HiFi II M-MLV (H-) (Glycerol-free) and RNase-free Water, and place on ice for later use.
2. Prepare the reaction system according to the following table, the total volume is 15 μL.

Reagent	15 μL Reaction	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	to 15 μL	

3. Incubate at 70 °C for 10 minutes and take a quick ice bath for 2 minutes.
4. Centrifuge briefly to collect the solution from the wall to the bottom of the tube.
5. Add 4 μL 5×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. It is not provided in this kit, if necessary, you can order it separately from our company. Cat.No.: CW0596.
6. Gently pipette to mix. If Oligo-DT Primers or Specific Primers are used for reverse transcription, incubate for 2 minutes at 42 °C. If Random Primers are used, incubate at 25 °C for 10 minutes.
7. Add 1 μL HiFi II M-MLV (Glycerol-free) (200 U/μL) to the tube, mix it gently with a pipette, 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. The reverse transcription products can be directly used for PCR reaction and fluorescence quantitative PCR reaction, or stored at -20 °C for six months. For long-term storage, it is recommended to store at -80 °C after packing. Repeated freeze-thaw should be avoided.

Version: 03/2024

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

Cat. No. : CW3353S (10000 U)
CW3353M (200 kU)
CW3353L (2000 kU)

Storage Conditions: -30 ~ -15 °C storage, dry ice transportation.
Avoid repeated freeze-thaw.

Components

Component	CW3353S 10000 U	CW3353M 200 kU	CW3353L 2000 kU
HiFi II M-MLV (H-) Reverse Transcriptase (Glycerol-free) (200 U /μL)	50 μL	1 mL	10 mL
5×SuperRT Buffer	1 mL	10 mL	100 mL

Introduction

HiFi II M-MLV (H-) Reverse Transcriptase (Glycerol-free) is a glycerol-free version of HiFi II M-MLV(H-)Reverse Transcriptase (CW0743). It is a reverse transcription enzyme in which the mutant M-MLV gene is recombined and expressed by *E. coli* engineering bacteria. The enzyme cancatalyze the polymerization of complementary DNA using RNA or DNA: RNA hybrid chain as template. The mutated HiFi II M-MLV(H-) reverse transcriptase lacks RNase H activity, reducing RNA degradation during reverse transcription and making it easier to obtain 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, preparation of full-length cDNA library and the application of freeze-dried RT-PCR products. This product does not contain freeze-dried forming components, and it can be customized when applied to freeze-dried products.

Activity Definition

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

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.

Precautions

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross contamination 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, it should be treated with 0.1% DEPC (diethyl pyrocarbonate) aqueous 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 gently to avoid foaming, and use after a short centrifugation. The enzymes involved should be put back to -15 °C or below 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. It is not provided in this kit, if necessary, you can order it separately from our company. Cat.No.: CW0596.

Protocol

Note:

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

i. Reverse transcription procedure:

1. Thaw the RNA templates, primers, dNTP Mix, 5×SuperRT Buffer, HiFi II M-MLV (H-) (Glycerol-free) and RNase-free Water, and place on ice for later use.
2. Prepare the reaction system according to the following table, the total volume is 20 μL.

Reagent	20 μL Reaction	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×SuperRT Buffer	4 μL	1 ×
HiFi II M-MLV (H-) (Glycerol-free) (200 U /μL)	0.5-1 μL	
RNase-Free Water	to 20 μL	

Note:

If the initial amount of RNA is less than 50 ng, RNA enzyme inhibitor (RNasin) is recommended. It is not provided in this kit, if necessary, you can order it separately from our company. Cat.No.: CW0596.

3. Vortex to mix, briefly centrifuge to collect the solution from the tube walls to the bottom of the tube.
4. Incubate at 55 °C for 1-30 minutes and at 85 °C for 5 minutes. After the reaction, centrifuge briefly and cool on ice.
5. The reverse transcription products can be directly used for PCR reaction and fluorescence quantitative PCR reaction, or stored at -20 °C for six months. For long-term storage, it is recommended to store at -80 °C after packing. Repeated freeze-thaw should be avoided.