Reagent	20 uL Reaction System	Final conc.
dNTP Mix, 2.5 mM Each	4 μL	500 μM Each
Primer Mix	2 μL	
RNA Template	X μL	1 ng-5 μg
RNase-Free Water	up to 13 μL	

3. Incubate at 70 °C for 10 min and quickly ice bath for 2 min.
4. Centrifuge briefly to collect the solution from the wall to the bottom of the tube.

Version: 01/2021

HiFi-MMLV cDNA Kit

Cat. No. : CW0744S (25 rxn)
CW0744M (100 rxn)

Shipping and Storage : -20°C

Components

Component	CW0744S 25 rxn	CW0744M 100 rxn
HiFi-MMLV, 200 U/μL	25 μL	100 μL
5×RT Buffer	120 μL	500 μL
Primer Mix	60 μL	240 μL
dNTP Mix, 2.5 mM Each	120 μL	500 μL
DTT, 0.1 M	60 μL	240 μL
RNase-Free Water	1 mL	1 mL

Principle

This product is a cDNA first chain synthesis kit specially prepared for the first step experiment of two-step RT-PCR. This product package contains all reagents required for reverse transcription from RNA template to the first strand of cDNA, including HiFi-MMLV reverse transcriptase, reaction buffer, primer, dNTP, etc. The loss of activity of HiFi-MMLV reverse transcriptase H reduced the degradation of RNA in the reverse transcriptase reaction , making it easier to obtain full-length cDNA. HiFi-MMLV reverse transcriptase has the characteristics of high thermal stability, which can increase the yield of cDNA and is convenient to use. The system has high compatibility for subsequent PCR and quantitative PCR tests, compatible with various PCR reactions and DNA polymerase.

Product Feature

- RNase H- : By mutating HiFi-MMLV reverse transcriptase, the activity of RNase H was lost, making it easier to obtain full-length cDNA.
- Easy to use: The kit contains all reagents required for reverse transcription except RNA templates.

Cautions

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross-contamination in experiments. It is recommended that RNA manipulation be carried out in special areas, using special instruments and consumables, and that operators wear masks and disposable gloves and change gloves frequently.
2. Disposable plastic utensils should be used as far as possible in the experiment. If glassware is used, 0.1% DEPC (diethyl pyrocarbonate) aqueous solution should be treated at 37°C for 12 hours and autoclaved at 120°C for 30 minutes before use, or the glassware should be sterilized at 180°C for 60 minutes after dry heat. The sterile water used in the experiment should be autoclaved with 0.1% DEPC.
3. Please mix all reagents in this kit upside down gently before use, avoid foaming as far as possible, and use after a short centrifugation. The enzymes involved should be returned to -20°C as soon as possible after use to avoid repeated freeze-thaw.
4. RNA enzyme inhibitors (RNasin) are recommended if the initial RNA amount is less than 50ng. This kit is not provided. If necessary, you can order it separately from our company. Cat. No.: CW0596.

Procedure

Note: A reaction system of 20 μl can be established with 10 ng-5 μg total RNA. If the amount of total RNA is greater than 5 μg, scale up the reaction system.

i Reverse transcription procedure:

1. Place the RNA template, primer, dNTP Mix, DTT, RT Buffer, HiFi-MMLV and RNase-Free Water were dissolved on ice for later use.
2. The reaction system was formulated according to the following table with a total volume of 20 μl.

Reagent	20 uL Reaction system	Final conc.
dNTP Mix, 2.5 mM Each	4 μL	500 μM Each
Primer Mix	2 μL	
RNA Template	X μL	1 ng-5 μg
5×RT Buffer	4 μL	1×
DTT, 0.1 M	2 μL	10 mM
HiFi-MMLV, 200 U/μL	1 μL	
RNase-Free Water	up to 20 μL	

Note: 1) RNA enzyme inhibitors (RNasin) are recommended if the initial RNA amount is less than 50ng. This kit is not supplied. If necessary, you can order it separately from our company. Cat. No.: CW0596.

2) Primer Mix is composed of Oligo(dT) and Random Primer.

3. Vortex mixing and temporary centrifugation, so that the solution on the wall of the tube collected to the bottom of the tube.
4. Incubate at 42°C for 30-50 minutes, then at 85°C for 5 minutes. At the end of the reaction, centrifuge briefly the product is briefly centrifuged and placed on ice to cool.
5. The retroproducts can be directly used for PCR reactions and fluorescence quantitative PCR reactions, or stored at -20°C for long term.

ii If reverse transcription efficiency is low, or the RNA template has a complex secondary structure and high GC content, the following steps are recommended:

1. lace the RNA template, primer, dNTP Mix, DTT, RT Buffer, HiFi-MMLV and RNase-Free Water were dissolved on ice for later use.
2. The reaction system was formulated according to the following table with a total volume of 13 μL.