Version: 01/2024

II. Reverse transcription reaction:

1. Set up the reaction on ice according the following table. To ensure the accuracy of reaction mixture preparation, first prepare a pre-mixture system in a quantity equal to the number of reactions plus 2. Add 10 μL of the prepared pre-mixture to the reaction tube from Step 1.

Reagent	20 μL Reaction
The reaction solution from step I	10 μL
5×HiFiScript RT Master Mix	4 μL
RNase-Free Water	6 μL

2. Vortex to mix well; Briefly centrifuge to collect all the solution to the bottom of the tube.
3. Reaction condition of cDNA synthesis: Incubate at 37°C for 15 minutes then incubate at 85°C for 5 seconds.
4. After the reaction is done, briefly centrifuge, then put on ice. For long time storage, please put it in -20°C.

HiFiScript gDNA Removal RT MasterMix

Cat. No. : CW2020S (10 rxns)
CW2020M (100 rxns)

Storage Condition: -20°C.

Components

Component	CW2020S (10 rxns)	CW2020M (100 rxns)
10×gDNA Remover Mix	10 μL	100 μL
5×HiFiScript RT MasterMix	40 μL	400 μL
RNase-Free Water	0.5 mL	1.5 mL

Introduction

This product is a kit for reverse transcription after removal of genomic DNA. The kit removes genomic DNA in 2 minutes at 42°C. Meanwhile, because the reverse transcription reagents contain components that inhibit gDNA Remover, the sample processed by gDNA remover can be directly used for reverse transcription reaction to synthesize cDNA.

This kit contains a novel high-performance reverse transcriptase HiFiScript. 5×HifiScript RT MasterMix contains all the components needed for reverse transcription. The novel mutation site greatly enhances the transcriptional activity of the enzyme. The reverse transcription reaction can be completed in 15 minutes.

Product Features

1. Rapid genomic DNA deletion: With the gDNA remover, it takes only 2 minutes to remove genomic DNA.
2. Rapid reverse transcription: It takes only 15 minutes to obtain the first strand of cDNA.
3. Easy to use: RT MasterMix contains all the components needed for reverse transcription and ready to use.
4. High sensitivity: pg of total RNA or mRNA can be used as template.
5. High efficiency of reverse transcription efficiency: the novel mutation site enhancers the activity of the enzyme, to increase the yield of cDNA.

Precautions

1. RNase contamination should be avoided during operation to prevent RNA degradation or cross-contamination in experiments. We suggest that the RNA experiments should be performed in a specialized area with specialized equipment and consumables. The operator should wear a mask and disposable gloves and change gloves frequently.
2. Try to use disposable plastic containers. If glassware is used, it should be treated at 37°C for 12 hours with 0.1% DEPC and autoclaved at 120°C for 30 minutes before use, or glassware is dry heat sterilized at 180°C for 60 minutes before use. Sterile water used in the experiment should be treated with 0.1% DEPC, then be autoclaved.

3. The reaction should be set up on ice to prevent RNA degradation. The enzymes should be returned to -20°C as soon as possible after use to avoid repeated freezing and thawing.

Protocol

Thaw the template RNA on ice; the kit components should be immediately placed on ice after thawing at room temperature. Each solution should be vortexed to mix, and centrifuge briefly before use.

I. Genomic DNA removal reaction:

1. Set up the reaction according to the following table, and the total volume is 10 μL.To ensure the accuracy of reaction mixture preparation, first prepare a pre-mixture system in a quantity equal to the number of reactions plus 2. Then, distribute it into each reaction tube before adding the RNA. samples.

Reagent	10 μL Reaction
10x gDNA Remover Mix	1 μL
RNA Template	10 pg-1 μg
RNase-Free Water	Up to 10 μL

Note: if the total RNA is more than 1 μg, please scale up the reaction volume accordingly.

2. Vortex to mix well; Briefly centrifuge to collect all the solution to the bottom of the tube.
3. Incubate at 42°C for 2 minutes (if at room temperature, it can be extended to 30 minutes).
4. After the reaction is done, briefly centrifuge, then put on ice.