Version: 12/2023

HiFiScript gDNA Removal cDNA Synthesis Kit

Cat. No. : CW2582M (100 rxns)
Storage Condition: -20 °C.

Components

Component	CW2582M (100 rxns)
gDNA Eraser	50 μL
10x gDNA Erase Buffer	120 μL
HiFiScript, 200 U/μL	100 μL
5xScriptRT Buffer	500 μL
Primer Mix	120 μL
RNase-Free Water	2x1 mL

Reagent	20 μL Reaction
The reaction solution from step I	10 μL
HiFiScript, 200 U/μL	1 μL
Primer Mix[1)]	1 μL
5x ScriptRT Buffer	4 μL
RNase-Free Water	4 μL

Note: 1) Oligo-dT Primer or Gene Specific Primer should be chosen according to the experimental requirements. For 20 ul reaction, it is recommended to use 50 pmol Oligo-dT or 2 pmol Gene Specific Pimer.

2. Vortex to mix well; Briefly centrifuge to collect all the solution to the bottom of the tube.
3. Reaction condition of cDNA synthesis:
   1) for downstream qPCR experiment, incubate at 42°C for 15 minutes then incubate at 85°C for 5 minutes.
   2) for regular PCR, incubate at 42°C for 30-50 minutes then incubate at 85°C for 5 minutes.

   Note: for templates with complex secondary structure, or high GC content, increase the temperature for reverse transcription to 50°C to increase the reverse transcription efficiency.
4. After the reaction is done, briefly centrifuge, then put on ice.For long time storage, please put it in -20°C.

   Note: for real-time PCR reaction, the volume of reverse transcription product should NOT exceed the 1/10 volume of the total PCR reaction.

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 Eraser, the sample processed by gDNA Eraser can be directly used for reverse transcription reaction to synthesize cDNA.

This kit is equipped with the novel and efficient reverse transcriptase, HiFiScript, featuring significantly improved transcription activity due to innovative mutation sites. The efficiency and yield of the first-strand cDNA synthesis are enhanced, allowing the synthesis of the first strand with pg-level total RNA or mRNA. If the reverse transcription product, is used for downstream fluorescence quantitative detection, the reverse transcription reaction can be completed at 42 °C for 15 minutes. This kit is suitable for first-strand cDNA synthesis and subsequent applications such as RT-PCR, RT-qPCR, and preparation of full-length cDNA libraries.

Product Feature

Rapid genomic DNA deletion, rapid reverse transcription, high sensitivity, high efficiency of reverse transcription.

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 done in specialized area with specialized equipment and consumables. The operator should wear a mask and disposable gloves and change gloves frequently.
2. 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.
3. The reaction volume can be scaled up, and a maximum of 1 μg of total RNA can be used in a 10 μL reaction.
4. The Primer Mix is made up with Oligo(dT) and Random primer. Oligo-dT Primer or Gene Specific Primer should be chosen according to the experimental requirements.
5. If the starting RNA is less than 50 ng, it is recommended to add the inhibitor of RNAase (RNasin). This kit does not include it, and it can be ordered separately if needed (Cat. Num.: CW0596).
6. For RNA templates with complex secondary structures, it is recommended to incubate the template RNA for 5 minutes at 65°C first, then place it on ice immediately, and centrifuge briefly for further processing.

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 prior to 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
10× gDNA Eraser Buffer	1 μL
gDNA Eraser	0.5 μL
RNA Template	10 pg-1 ug
RNase-Free Water	Up to 10 μL

Note: if the total RNA is more than 1 ug, please scale up the reaction volume accordingly. If the starting RNA is less than 50 ng, it is recommended to add the inhibitor of RNAase (RNasin: CW0596).

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.

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.