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LiScript™ Fast RT Master Mix (+gDNA wiper)

Cat. #: M0039 Size: 100 rxns

Introduction

LiScript™ Fast RT Master Mix (+gDNA wiper) 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 LiScript reverse transcriptase. 5× LiScript RT MasterMix contains all the components needed for reverse transcription. The novel mutation site greatly enhances the transcriptional activity of the enzyme. The efficiency and yield of cDNA first-strand synthesis are higher, and the first strand of cDNA can be synthesized using pg total RNA or mRNA. If the cDNA is used for downstream qPCR, the reverse transcription reaction can be completed in 15 minutes. This kit is suitable for the synthesis of first-strand cDNA and subsequent RT-PCR, RT-qPCR, and construction of full-length cDNA libraries.

Package Information

Components	M0039
10× gDNA Remover Mix	100 μl
5× LiScript™ RT Master Mix	400 μl
RNase-Free Water	1.5 ml

Storage

Store at -20°C

Preparation before the experiment and 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.

4. 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.

1. Genomic DNA removal reaction:

Set up the reaction according to the following table, and the total volume is 10 μl.

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

Note:
1) If the total RNA is more than 1 μg, please scale up the reaction volume accordingly. If the starting RNA is less than 50 ng, it is recommended to add the inhibitor ofRNAase.

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.

2. Reverse transcription reaction:

2.1 Set up the reaction on ice according the following table:

Reagent	20 μl Reaction
The reaction solution from step 1	10 μl
5× LiScript™ 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 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.

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