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LiScript™ First Strand cDNA Synthesis Kit (+gDNA Wiper)

Cat. #: M0028 Size: 100 rxns

Introduction

LiScript™ First Strand cDNA Synthesis Kit (+gDNA Wiper) contains a novel high-performance reverse transcriptase. 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. 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 at 42°C for 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	M0028
gDNA Eraser	50 µl
10× gDNA Erase Buffer	120 µl
LiScript™ RT, 200 U/µl	100 µl
5× ScriptRT Buffer	500 µl
Primer Mix	120 µl
RNase-Free Water	2×1 ml

Storage

All the components should be stored at -20°C

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.

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.

Reagent	10 µl Reaction
10× gDNA Eraser Buffer	1 µl
gDNA Eraser	0.5 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. If the starting RNA is less than 50 ng, it is recommended to add the inhibitor of RNAase.

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.

Reagent	20 µl Reaction
The reaction solution from step I	10 µl
LiScript™ RT, 200 U/µl	1 µl
Primer Mix*	1 µl
5× ScriptRT Buffer	4 µl
RNase-Free Water	4 µl

Note: Oligo-dT Primer or Gene Specific Primer should be chosen according to the experimental requirements. For 20 µl 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.

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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.