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LiScript™ First-Strand cDNA Synthesis Kit

Cat. #: M0029 Size: 100 rxn

Introduction

LiScript™ First-strand cDNA Synthesis Kit is a first-strand cDNA synthesis kit designed for the first step of two-step RT-PCR. The LiScript reverse transcriptase used in the kit is a novel high-efficiency reverse transcriptase derived from M-MLV. The novel mutation site greatly enhances the transcriptional activity of the enzyme. The efficiency and yield of cDNA first-strand synthesis are higher, and this kit can be used with as little as pg level of total RNA or mRNA.

The point mutation eliminates RNase H activity, improves the elongation ability of the enzyme, and effectively improves the affinity of the reverse transcriptase to the RNA template, resulting in a first strand cDNA of up to 12 kb. Optimized RT Buffers allow a wider range of applications for reverse transcriptase and are highly compatible with subsequent PCR and quantitative PCR experiments. The kit is equipped with all reverse transcription reagents and is easy to use. It 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	M0029
LiScript Enzyme Mix (200 U/μl)	200 μl
5× RT Buffer	500 μl
Primer Mix	240 μl
dNTP Mix, 2.5 mM each	500 μl
DTT, 0.1 M	240 μl
RNase-Free Water	1 ml

Storage

This product should be stored 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 done in 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. All the reagents in this kit should be mixed upside down gently before use, try to avoid foaming, and use after a brief centrifugation. The enzymes should be returned to -20°C as soon as possible after use to avoid repeated freezing and thawing.

4. If the amount of the starting RNA is less than 50 ng, it is recommended to add RNase inhibitor. This kit does not include it, and it can be ordered separately if needed.

Protocol

Note: a 20-μl reaction can be set up with 1 ng-5 μg of total RNA. If the total RNA is greater than 5 μg, scale up the reaction system.

Reverse transcription protocol

1. RNA template, Primer Mix, dNTP Mix, DTT, RT Buffer, LiScript, and RNase-Free water are thawed and placed on ice for use.

2. Prepare the reaction system according to the following table for a total volume of 20 μl.

Reagent	20-μl reaction	Final Conc.
dNTP Mix, 2.5 mM each	4 μl	0.5 mM each
Primer Mix	2 μl	
RNA Template	x μl	50 pg-5 μg
5× RT Buffer	4 μl	1×
DTT, 0.1 M	2 μl	10 mM
LiScript Enzyme Mix	1 μl	
RNase-Free Water	Up to 20 μl	

Note:

1. If the amount of the starting RNA is less than 50 ng, it is recommended to add RNase inhibitor (RNasin). RNasin is not provided.

2. Primer Mix is formulated with Oligo(dT) and Random Primer.

3. Vortex to mix and briefly centrifuge to collect the solution to the bottom of the tube.

4. cDNA synthesis reaction condition:

   1) If qPCR detection is performed downstream, incubate at 42°C for 15 minutes, then incubated at 85°C for 5 minutes.

   2) For downstream regular PCR, incubate at 42°C for 30-50 minutes, then incubate at 85°C for 5 minutes.

   3) For templates with complex secondary structures or high GC contents, the reverse transcription temperature can be increased to 50°C to enhance the reverse transcription efficiency.

5. After the reaction is done, briefly centrifuge and placed it on ice to cool down.

6. Reverse transcription products can be used directly in PCR and qPCR reactions or placed at -20°C for long-term storage.

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If the efficiency of reverse transcription is low, or the secondary structure of the RNA template is complex and the GC content is high, the following protocol is recommended:

1. RNA template, Primer Mix, dNTP Mix, DTT, RT Buffer, LiScript and RNase-Free water are thawed and placed on ice for use.

2. Prepare the reaction system according to the following table for a total volume of 13 µl.

Reagent	20-µl reaction	Final Conc.
dNTP Mix, 2.5 mM each	4 µl	0.5 mM each
Primer Mix	2 µl	
RNA Template	x µl	50 pg-5 µg
RNase-Free Water	Up to 13 µl	

3. Incubate at 70°C for 10 minutes and quickly incubate on ice for 2 minutes.

4. Briefly centrifuge to collect the solution from the tube wall to the bottom of the tube.

5. Continue adding the following reagents to the above reaction solution:

Reagent	20-µl reaction	Final Conc.
5× RT Buffer	4 µl	1×
DTT, 0.1 M	2 µl	10 mM
LiScript Enzyme Mix	1 µl	
RNase-Free Water	Up to 20 µl	

Note:
1) If the amount of the starting RNA is less than 50 ng, it is recommended to add RNase inhibitor (RNasin). RNasin is not provided.

2) Primer Mix is formulated with Oligo(dT) and Random Primer.
Reagent

6. cDNA synthesis reaction condition:

1) If qPCR detection is performed downstream, incubate at 50°C for 15 minutes, then incubate at 85°C for 5 minutes.

2) For downstream regular PCR, incubate at 50°C for 30-50 minutes, then incubate at 85°C for 5 minutes.

7. After the reaction is done, briefly centrifuge and place it on ice to cool down.

8. Reverse transcription products can be used directly in PCR and qPCR reactions, and the amount added should be less than 1/10 of the PCR reaction system. Or the reverse transcription products can be placed at -20°C for long-term storage.