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9610 Medical Center Drive, Rockville, MD 20850, USA

LiScript™ Reverse Transcriptase

Cat. #: M0022 Size: 10,000 U

Introduction

For RNA with complex secondary structure, the reverse transcription reaction temperature can be raised to 50 ~ 55°C. In addition, this product has superior continuous synthesis ability and inhibitor tolerance.

Package Information

Components	M0022
5× LiScript™ Buffer	500 µl
LiScript™ Reverse Transcriptase	50 µl, 200 U/µl

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 10 min at 37°C with Poly (rA)·Oligo (dT) as the template/primer.

Source

Recombinant *E. coli*.

Notes

Prevent RNase contamination

Keep the experiment area clean. Wear disposable gloves and masks, and use RNase-free centrifuge tubes and tips.

Primer selection

1. For PCR

For eukaryotic RNA templates, use Oligo dT primer to obtain the highest yield of full-length cDNA by pairing with 3' Poly A of eukaryotic mRNA.

Gene Specific Primers (GSP) has the highest specificity. If GSP fails in the 1st strand cDNA synthesis, Oligo $(dT)_{20}VN$ or Random hexamers can be used for reverse transcription.

Random hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be used as the template of Random hexamers. Random hexamers can be used as primers, when Oligo $(dT)_{20}VN$ or GSP can not effectively guide cDNA synthesis for the target region has complex secondary structure and high GC content, or the template is prokaryotic origin.

2. For qPCR

Use the mixture of Oligo dT and random hexamers. In this way, the cDNA synthesis efficiency of each region of the mRNA can be the same, which helps to improve the authenticity and repeatability of the quantitative results.

Protocol

For PCR

1. RNA Denaturation

Mix the following components in an RNase-free centrifuge tube:

RNase free ddH_2O	to 10 µl
Total RNA	10 pg - 5 µg
or Poly A+ RNA	10 pg - 500 ng

Incubate at 65°C for 5 min and then chill on ice immediately for 2 min.

Note: The denaturation step helps to open the secondary structures to improve the first strand cDNA yield. For cDNA fragment longer than 3 kb, please do not ignore the denaturation step.

2. Preparation of reaction solution for 1st strand cDNA synthesis

Mixture of Step 1	10 µl
5× LiScript™ Buffer	4 µl
dNTP Mix (10 mM each)	1 µl
LiScript™ Reverse Transcriptase (200 U/µl)	1 µl
RNase inhibitor (40 U/µl)	1 µl
Oligo $(dT)_{20}VN$ (50 µM) or Random hexamers (100 µM) or Gene Specific Primers (2 µM)	1 µl
RNase-free ddH_2O	2 µl

Gently pipette up and down several times to mix thoroughly.

3. Reaction Program

25°C*	5 min
37°C**	45 min
85°C	5 sec

* This step is required only when using the Random hexamers. Please omit this step when using Oligo $(dT)_{20}VN$ or GSP.

** For templates with complex secondary structure or high GC content, the temperature can be increased to 50°C, which will benefit the yield.

The product can be used for PCR immediately or be stored at -20°C for 6 months. It is recommended to store in aliquots at -70°C for long term storage. cDNA should avoid repeated freezing and thawing.

FOR RESEARCH USE ONLY

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For qPCR

1. Preparation of reaction solution for 1st strand cDNA synthesis
Mix the following components in an RNase-free centrifuge tube:

RNase-free ddH_2O	to 20 µl
5× LiScript™ Buffer	4 µl
dNTP Mix (10 mM each)	1 µl
LiScript™ Reverse Transcriptase (200 U/µl)	1 µl
RNase inhibitor (40 U/µl)	1 µl
Oligo $(dT)_{20}$VN (50 µM)	1 µl
Random hexamers (100 µM)	1 µl
Total RNA	10 pg - 1 µg
Poly A+ RNA	10 pg - 100 ng

Gently pipette up and down several times to mix thoroughly.

2. Reaction Program

37°C*	15 min
85°C	5 sec

* For templates with complex secondary structure or high GC content, the temperature can be increased to 50°C, which will benefit the yield.

The product can be used for qPCR immediately or be stored at -20°C for 6 months. It is recommended to store in aliquots at -70°C for long term storage. cDNA should avoid repeated freezing and thawing.