LifeSct.com
Life Scientists' Service Center
Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
14323 Woodcrest Dr., Rockville, MD 20853

LiScript™ 1st Strand cDNA Synthesis Kit

Cat. #: M0031 Size: 50/100 rxns

Introduction

LiScript™ 1st strand cDNA Synthesis Kit contains all components needed to synthesize high-quality 1st strand cDNA. Reaction products are applicable to subsequent PCR, qPCR and PCR cloning. 2× RT Mix contains optimized buffer system and dNTP; Enzyme Mix contains LiScript® Reverse Transcriptase and RNase inhibitor. Oligo $(dT)_{18}$ and Random hexamers can be chosen as primers for reverse transcription as needed. Compare with Oligo dT_{18}, Oligo $dT_{23}VN$ in Kit has stronger anchoring ability for Poly A+ RNA, which makes the reverse transcription efficiency is higher. Choose Oligo $dT_{23}VN$ accordingly, Random hexamers or gene specific primers for reverse transcription primer.

Package Information

Components	M0026-05 (50 rxn/ 20 µl/ rxn)	M0026-25 (100 rxn/ 20 µl/ rxn)
RNase free ddH_2O	1 ml	1 ml
2× RT Mix*	500 µl	1 ml
LiScript™ Enzyme Mix**	100 µl	200 µl
Oligo dT_{18} (50µM)	50 µl	100 ul
Random hexamers (50 ng/ µl)	50 µl	100 ul

* Contain 1 mM each dNTP. ** Contain RNase inhibitor.

Storage

This product should be stored at -20°C for 12 months.

Protocol

Guidelines for PCR reaction

1. RNA template denaturation

Set up the following mixture in RNase free centrifuge tube

RNase free ddH_2O	to 8 µl
Oligo$(dT)_{23}VN$ (50 µM)	1 µl
or Random hexamers (50 ng/µl)	1 µl
or Gene Specific Primers (2 µM)	1 µl
Total RNA	10 pg - 5 µg
Poly (A)+ RNA	10 pg - 500 ng

65°C heated 5 min, placed on ice rapidly quenched, and allowed to stand on ice for 2 min. RNA template denaturation help open the secondary structure can improve the yield of first strand cDNA to a large extent. For longer than 3 kb cDNA fragment, do not omit the denaturation step

2. Preparation of a first strand cDNA synthesis reaction

Mixture	8 ul
5× LiScript™ Enzyme Mix	2 µl
2× RT Mix	10 ul

Mix gently with a pipette.

3. Under the following conditions of the first strand cDNA synthesis reaction

25°C*	5 min
50°C**	45 min
85°C	5 min

* This step is required only when using the Random hexamers; using Oligo $dT_{23}VN$ or Gene Specific Primer omits this step.
** If the template has a complicated secondary structure, the reaction temperature was raised to 55°C, helps to increase production.

The product can be used in PCR reactions immediately, or at -20°C, and used within six months; after long-term storage is recommended aliquots at -80°C. cDNA Avoid repeated freezing and thawing.

Guidelines for qPCR reaction

1. RNA template denaturation

Set up the following mixture in RNase free centrifuge tube

RNase free ddH_2O	to 20 µl
2× RT Mix	10 µl
LiScript™ Enzyme Mix	2 µl
Oligo $dT_{23}VN$ (50ng/ µM)	1 µl
Random hexamers (50ng/ µl)	1 µl
Total RNA	10 pg - 51 µg
Poly (A)+ RNA	10 pg - 100 ng

2. Under the following conditions of the first strand cDNA synthesis reaction

25°C	5 min
50°C*	15 min
85°C	5 min

* For templates with complex secondary structure, raise temperature to 55°C to improve cDNA increment.

The product can be used in PCR reactions immediately, or at -20°C, and used within six months; after long-term storage is recommended aliquots at -80°C. cDNA Avoid repeated freezing and thawing.

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Quality Control

All components have been tested containing no exonuclease, endonuclease and RNase residues.

Functional test

1. Taking 500 ng Hela cell total RNA as template and Oligo $dT_{23}VN$ as primer, react for 45 min at 50°C,. Take 1/10 of cDNA products to carry out PCR amplification of DNCH gene. After agarose gel electrophoresis and EB staining, a clear 20.0 kb band can be detected..

2. Taking 100 pg Hela cell total RNA as template and Oligo $dT_{23}VN$ as primer, react for 30 min at 50°C,. Take 1/10 of cDNA products to carry out PCR amplification of β-actin gene. After agarose gel electrophoresis and EB staining, a clear 550 bp band can be detected.

3. Taking 500 ng Hela cell total RNA as template and Oligo $dT_{23}VN$ as primer, react for 45 min at 55°C,. Take 1/10 of cDNA products to carry out PCR amplification of β-actin gene. After agarose gel electrophoresis and EB staining, a clear 4.8 kb (GC-rich) band can be detected.

4. Taking 1 pg-1 μg Hela cell total RNA as template and Oligo $dT_{23}VN$ as primer, react for 30 min at 50°C,. Performance test of RT-qPCR. By 6 orders of magnitude of the template amount on the numerical value of Ct standard curve, R2>0.990, slope between -3.20 to -3.60.

Notes

Prevent RNase contamination

Please keep the experiment area clean; operations are required to wear clean gloves, masks, centrifuge tubes, tips and other supplies used in the experiments required to ensure RNase free.

Primers Choosing for PCR

- Oligo $(dT)_{23}VN$ hybridizes at high efficiency to the 3´ poly(A) region present in most mature eukaryotic mRNA.. It is the first choice for most cases and generally the full-length cDNA of the highest yield can be obtained.

- Random hexamers is of lowest specificity. All RNA, including mRNA, rRNA and tRNA can be templates of Random hexamers. When the target area of RNA has complex secondary structure or is GC-rich, and Oligo $(dT)_{18}$ or gene specific primers (GSP) cannot effectively synthesize cDNA, Random hexamers can be used.

- Gene specific primer (GSP) has the highest specificity. But under some circumstances, GSP used for PCR reaction cannot effectively synthesize cDNA. Then use Oligo $(dT)_{18}$ instead and try again.

Primers Choosing for qPCR

The Oligo $dT_{23}VN$ mixed with Random hexamers, which allows each region of mRNA at the same efficiency can lead to cDNA synthesis, helps to improve the repeatability of quantitative results.