Ver.23111

StarScript First Strand cDNA Synthesis Kit

Cat.No.ODR42

Description: StarScript First Strand synthesis kit contains StarScript RTase which is the mutated form of M-MLV Reverse Transcriptase, rendering the enzyme thermostable with no RNase H activity. It can retain 100% activity at 50℃; also retains more than 80% activity even at 55℃.

Product contents

Contents	Volume
StarScript RTase (200U/μL)	5000U
5X StarScript Buffer (Included 50mM DTT mix)	200μL
10mM dNTP mix	50μL
RNase-free Water	600μL
RNase inhibitor	50μL
Random hexamer (2.8nmol)	Lyophilized Dissolve in 56μL nuclease free water to obtain 50μM stock.
Oligo(dT)$_{18}$ (2.8nmol)	Lyophilized Dissolve in 56μL nuclease free water to obtain 50μM stock.

Note: Prepare aliquots of Oligo(dT)$_{18}$ and Random Hexamer to avoid multiple freeze thaw cycles.

Storage

- Store at –20°C

Features

RNase H activity	Negative
Temperature of cDNA synthesis	At 42-55ºC
Synthesis length	< 14 kb
Synthesis Primer	Random primer, Oligo(dT)$_{18}$,Gene specific
Expiration date (at -20ºC)	2 years

Protocol

First Strand cDNA synthesis (reaction volume 20µL)

1. Prepare the following mixture in a PCR tube.

RNA	10ng –1µg total RNA 10ng – 0.5µg mRNA	- µg
Primer	1µL Oligo(dT)$_{18}$ /1µL Random hexamer or 0.5µL Oligo(dT)$_{18}$ + 0.5µL Random hexamer or 1µL Gene-specific Primer (15 – 20µM)	
dNTP (10mM)		1µL
Make up the volume with RNase-free Water to		14.5µL

2. Heat the mixture at 65ºC for 5 minutes and cool down immediately on ice for 1 minute. Spin down the tube by centrifugation briefly.
   Note: Use heating block or PCR machine. DO NOT use water bath.

3. In the same tube, add the following reaction mixture:
   (Total reaction volume- 20µL)

Reagents	Volume
5X StarScript Buffer	4µL
StarScript RTase	0.5µL
RNase inhibitor	1µL

4. Mix gently.
   Note: For random hexamer, incubate the combined reaction mixture at 25ºC for 5 minutes and then proceed with the next step. For oligo(dT)$_{18}$ or gene-specific primers, directly proceed with the next step.

5. Incubate at 50ºC for 10 minutes.

6. Inactivate the reaction mixture by heating at 95ºC for 5 minutes.
   Note: Use heating block or PCR machine. DO NOT use water bath.

7. The product can be used for PCR immediately or stored at -20ºC.
   Note: As a recommendation; cDNA product should compose 10% of total reaction volume of PCR.