Ver.23.04

cDNA synthesis kit

Cat.No. ODR23

Description: RTase used in this kit is a thermostable mutant of M-MLV reverse transcriptase (RNase H) which is a recombinant type and purified from *E.coli*. This mutant type of RNase H enables more efficient cDNA synthesis.

Product contents

Contents	Volume (100 Preps)
RTase (200U/μL)	10000U
5X RT Reaction Buffer (Included 50mM DTT mix)	400μL
10mM dNTP	100μL
RNase-free Water	1.2μL
Random hexamer (2.8nmol)	Lyophilized (×2 tubes) Dissolve the contents of one tube in 56μL nuclease free water.
Oligo $(dT)_{18}$ (2.8nmol)	Lyophilized (×2 tubes) Dissolve the contents of one tube in 56μL nuclease free water.

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

Storage

- Store at –20°C

Features

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RNase H activity	RNase H 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 (Total 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		15.5µL

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. Add the following components to the same tube:
   (Total reaction volume - 20µL)

Reagents	Volume
5X RT Reaction Buffer	4µL
RTase	0.5µL

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 60 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.