

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 (50 Preps)
RTase (200U/ μ L)	5000U
5X RT Reaction Buffer (Included 50mM DTT mix)	200 μ L
10mM dNTP	100 μ L
RNase-free Water	600 μ L
Random hexamer (2.8nmol)	Lyophilized Dissolve in 56 μ L nuclease free water
Oligo (dT) ₁₈ (2.8nmol)	Lyophilized Dissolve in 56 μ L 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 $42-55^{\circ}\text{C}$
Synthesis length	< 14 kb
Synthesis Primer	Random primer, Oligo (dT) ₁₈ , Gene specific
Expiration date (at -20°C)	2 years

Protocol

First Strand cDNA synthesis (Total reaction volume - 20 μ L)

1. Prepare the following mixture in a PCR tube.

RNA	10ng – 1 μ g total RNA 10ng – 0.5 μ g mRNA	- μ g
Primer	1 μ L Oligo (dT) ₁₈ / 1 μ L Random hexamer or 0.5 μ L Oligo (dT) ₁₈ + 0.5 μ L Random hexamer or 1 μ L Gene-specific Primer (15 – 20 μ M)	
	dNTP (10mM)	2 μ L
	Make up the volume with RNase-free Water to	15.5 μ L

- Heat the mixture at 65°C 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.

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

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

- Mix gently.
Note: For random hexamer, incubate the combined reaction mixture at 25°C for 5 minutes and then proceed with the next step. For oligo (dT)₁₈ or gene-specific primers, directly proceed with the next step.
- Incubate at 50°C for 60 minutes.
- Inactivate the reaction mixture by heating at 95°C for 5 minutes.
Note: Use heating block or PCR machine. DO NOT use water bath.
- The product can be used for PCR immediately or stored at -20°C.
Note: As a recommendation; cDNA product should compose 10% of total reaction volume of PCR.