

# 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/μL)	5000U
5X RT Reaction Buffer (Included 50mM DTT mix)	200μL
10mM dNTP	100μL
RNase-free Water	600μL
Random hexamer (2.8nmol)	Lyophilized Dissolve in 56μL nuclease free water
Oligo (dT) <sub>18</sub> (2.8nmol)	Lyophilized Dissolve in 56μL nuclease free water

**Note:** Prepare aliquots of Random Hexamer and Oligo (dT)<sub>18</sub> to avoid multiple freeze thaw cycles.

## Storage

- Store at -20°C

## Features

Features	
RNase H activity	RNase H negative
Temperature of cDNA synthesis	At 42-55°C
Synthesis length	< 14 kb
Synthesis Primer	Random primer, Oligo (dT) <sub>18</sub> , Gene specific
Expiration date (at -20°C)	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) <sub>18</sub> / 1μL Random hexamer or 0.5μL Oligo (dT) <sub>18</sub> + 0.5μL Random hexamer or 1μL Gene-specific Primer (15 – 20μM)	
	dNTP (10mM)	2μL
	Make up the volume with RNase-free Water to	15.5μL

2. 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.**

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 25°C for 5 minutes and then proceed with the next step. For oligo (dT)<sub>18</sub> or gene-specific primers, directly proceed with the next step.

5. Incubate at 50°C for 60 minutes.

6. Inactivate the reaction mixture by heating at 95°C 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 -20°C.

**Note: As a recommendation; cDNA product should compose 10% of total reaction volume of PCR.**