

# Reverse Transcriptase

## 1 Packing list

Components	HY-KE8004-10000U
Reverse Transcriptase (200 U/μL)	50 μL
5× RT Buffer	1 mL

## 2 Introduction

This product is a reverse transcriptase that clones and expresses the deletion mutant RNase H- of M-MuLV through genetic recombination technology. General wild-type M-MuLV (Moloney Murine Leukemia Virus) has the following activities:1) RNA-dependent DNA polymerase activity;2) DNA polymerase activity that depends on DNA;3) RNase H activity. Since RNase H can catalyze the degradation of RNA in DNA/RNA hybrids, the template RNA in RNA/DNA hybrids may be degraded during the synthesis reaction of the first strand of cDNA. This enzyme lacks RNase H activity and has strong extension ability. It can be used for longer cDNA synthesis, construction of high-proportion full-length cDNA libraries, and Real Time RT-PCR reactions.

## 3 Unit definition

Using Poly(rA)-Oligo(dT) as template/primer, the amount of enzyme required to incorporate 1 nmol of [3H] dTTP at 37°C for 10min is defined as 1 unit.

## 4 General Protocol

cDNA first-strand synthesis reaction system

- 1) After the reagents have melted, mix the components and centrifuge slightly before placing on ice.
- 2) Add the following reactants in order:

Components	Adding amount
Total RNA	0.1 ng-5 μg
poly(A) mRNA	10 pg
Specific RNA	0.01 pg
Oligo (dT) <sub>18</sub>	1 μL
Random N6	1 μL
Gene specific primers	15-20 pmol
5× RT Buffer	4 μL
RNase Inhibitor(40 U/μL)	1 μL
dNTPs(10 mM)	2 μL
Reverse Transcriptase	1 μL
Water(RNase-Free)	Up to 20 μL

3) 1) If the RNA template has high GC content or contains secondary structures, mix the RNA template and Water (RNase-Free) first, incubate at 65°C for 5min, cool on ice, and then add other components.

4) Mix gently and centrifuge. Incubate at 42°C for 15-30min. If the RNA template does not contain poly A structure, first incubate at 25°C for 5min, then incubate at 42°C for 15-30min. The reaction product can be used directly for PCR. If not used immediately, it should be stored at -20°C for less than one week. It is recommended to store it at -70°C for long-term storage.

Note: Experimental results suggest that this reverse transcriptase can complete a 1kb reverse transcription reaction in 15min.

## 5 Storage

-20°C, 1 years

## 6 Precautions

1. Bst DNA polymerase does not have 3' → 5' exonuclease activity.
2. For long-term storage, add 100 µg/mL BSA or 0.1% Triton X-100.
3. It is recommended that the reaction temperature should not exceed 70°C.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.