LaboPass[™] M-MuLV Reverse Transcriptase

Cat. No CMRT010 10,000 uni
Cat. No CMRT050 50.000 uni

10,000 unit (200 unit/µl) 50,000 unit (200 unit/µl)

Lot No.

Note: For laboratory use only

Description

LaboPass[™] M-MuLV Reverse Transcriptase is a recombinant form of the reverse transcriptase from the Moloney Murine Leukemia Virus (M-MuLV) which possesses enhanced cDNA synthesis activity and reduced RNase H activity. Reduction of RNase H activity enables higher yield of full-length cDNA transcripts and increased thermostability over wild type enzyme. The enzyme can synthesize cDNA using either RNA or single stranded DNA as a template. It is expressed in *E.coli* and purified with high purity.

Contents

• M-MuLV Reverse Transcriptase (200 unit/µI)

5X RT reaction buffer
dNTPs (Each 10 mM)

Store at -20°C

CMRT010

CMRT010

10,000 unit 50,000 unit 1 ml X 2 vials

0.1 ml

0. 5 ml

Applications

- First strand cDNA synthesis
- RT-PCR or qRT-PCR
- · cDNA labeling for microarray or other applications

Unit Definition

One unit is defined as the amount of enzyme that incorporates 1 nmole of dTTP into acid-insoluble material in 10 min at $37\,^{\circ}\text{C}$ using poly(A)-oligo(dT) as template-primer.

5X RT reaction buffer

 $250 \,\mathrm{mM}$ Tris-HCl (pH 8.3), $375 \,\mathrm{mM}$ KCl, $30 \,\mathrm{mM}$ MgCl $_2$, $25 \,\mathrm{mM}$ DTT

Storage Buffer

20 mM Tris-HCl (pH 7.5), 200 mM NaQ, 0.1 mM EDTA, 1 mM DTT, 0.01 % (v/v) NP-40, 50 % (v/v) Glycerol

Standard Protocol

1. Add the following components into a sterile, nuclease-free tube for each 20 µl reaction:

RNA template 10 pg to 5 µg according to target abundance Primer 100 pmole oligo(dT)₁₂₋₁₈ or random primer

10 to 20 pmole gene-specific primer

dNTPs (each 10 mM) 1 µl Nuclease-free water up to 14 µl

- 2. Heat for 5 min at 65 °C, quick chill on ice and briefly spin-down.
- 3. Add the following components to the above mixture and gently mix

5X RT reaction buffer 4 μ I RNase inhibitor (40 unit/ μ I)* 1 μ I M-MLV Reverse Transcriptase 1 μ I

*Addition of RNase inhibitor greatly improves yield of cDNA

- 4. Incubate at 42 °C for $30 \sim 60$ min.
- 5. Inactivate enzyme by heating at 70 °C for 15 min.
- 6. Store at -20 °C until use

