M-MLV Thermostable (H-)Reverse Transcriptase

M-MLV thermostable	50 µl
5X first-strand Buffer	200 µl

For research use only
Cat No: YT4503
Size: 10000 U (200U/µl)
Store at –20°C

Description :

Thermostable Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) is an engineered version of M-MLV reverse transcriptase with reduced RNase H activity increased thermal stability. This enzyme have a polymerase feature and uses single-stranded RNA or DNA in the presence of a primer to synthesize a complementary DNA strand. M-MLV thermostable enzyme is an ideal choice for higher cDNA yield from low starting amounts of RNA. The broad range of temperature up to 60°C delivers reverse transcription at your temperature of choice. The enzyme can generate first-strand cDNA up to 10 kb.

Features:

- Optimum reaction temperature is 37°C to 55°C
- Highest reaction temperature is 60°C
- produces higher yield of cDNA
- synthesis longer cDNA up to 10kb

Applications:

- RT-LAMP
- First-strand cDNA Synthesis
- One-step qRT-PCR

Storage Buffer

20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% NP-40, 50% glycerol

5xfirst-Strand Buffer

250mM Tris-HCl (pH 8.3 at 25°C), 375mM KCl,15mM MgCl2 50mM DTT

Protocol for first strand cDNA synthesis:

20-µl reaction volume can be used for 100pg of total RNA .

1. Add the following reagents into a sterile, nuclease-free nuclease-free tube on ice in the indicated order:

Template RNA	Total RNA or specific RNA mRNA	100pg-5µg 100pg-500ng
Primer	oligo (dT)18 primer(50µM) or Random hexamer primer(50µM)	1µl 1µl
DEPC-treated water		to 13.5µl
Total volume		13.5µl

2. Mix gently, centrifuge briefly and incubate at 70°C for 5 min. Chill on ice, spin down and place the vial back on ice.

3. Prepare the following cDNA Synthesis Mix, add the following components in the indicated order:

5x first-strand buffer	4µl
dNTPs(10 mM each)	1µl
RNasin (40U/µl)	0.5µl
M-MLV	1µl

4. Mix gently and centrifuge.

5.For oligo(dT)18, incubate for 60 min at 42°C. For random hexamer primed synthesis, incubate for 60 min at 37°C .

6. Terminate the reaction by heating at 70°C for 5 min. The reverse transcription reaction product can be used immediately in second strand cDNA synthesis reactions or stored at -20°C for less than a week. For longer storage, -70°C is recommended.