

## cDNA Synthesis Kit

**Cat No: YT4500**

**for research use only**

**Size: 50 tests**

**< store at -20°C >**

Contents:	
M-MLV (10,000 U) 200u/ µl	<b>50 µl</b>
5X first-strand Buffer	<b>200 µl</b>
Oligo(dT)18 primer	*
Random hexamer primer	**
RNasein (40u/µl)	<b>25 µl</b>
dNTP 10mM	<b>50 µl</b>

### Important Note :

**Primers are lyophilized.**

**Please add DEPC water used for Con.50 µM**

\* **Oligo(dT)18 primer : 60.96 µl**

\*\* **Random Hexamer : 184.48 µl**

### **Description :**

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) uses single-stranded RNA or DNA in the presence of a primer to synthesize a complementary DNA strand. This enzyme is isolated from E. coli expressing a portion of the pol gene of M-MLV on a plasmid. The enzyme is used to synthesize first-strand cDNA up to 5 kb.

### **Unit Definition :**

One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10 min at 37°C using poly(A) •oligo(dT) 25 as template-primer.

### **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 MgCl<sub>2</sub> 50mM DTT

### **Applications:**

Generation of first strand cDNA for use in:

- PCR, see Protocol for First-strand cDNA Synthesis;
- Second strand cDNA synthesis.
- DNA labeling.
- Real-time PCR;
- Analysis of RNA by primer extension.

### **Protocol:**

First-Strand cDNA Synthesis Using M-MLV RT

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

Template RNA	Total RNA Poly (A) mRNA Or specific RNA	0.1ng - 5µg 1 to 500 ng 1-5 µg
Prime	oligo (dT)18 primer(50µM) or Random hexamer primer(50µM)	1.0 µl 1.0 µl
DEPC-treated water		To 13.4 µl
Total Volume		13.4 µl

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	4 $\mu$ l
dNTPs(10 mM each)	1 $\mu$ l
RNasin (40U/ $\mu$ l)	0.5 $\mu$ l
M-MLV	1 $\mu$ l

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.