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یکتا تجهیز آزما

cDNA Synthesis Kit

Cat No: YT4500
Size: 50 tests

for research use only
< store at -20°C >

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

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 MgCl2 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 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 13.5 µl
Total Volume		13.5 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 µl
dNTPs(10 mM each)	1 µl
RNasin (40U/µl)	0.5 µl
M-MLV	1 µl

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.