M-MLV Reverse transcripatase（RNaseH-）

Description:

Moloney Murine Leukemia Virus Reverse Transcriptase(M-MLV RT) is isolated from recombinant E.coli strain expressing the gene of M-MLV. The recombinant protein is obtained by column purification.

M-MLV (RNase H^-) lacks the activity of RNase H for multiple point mutations. It has the same activity of DNA polymerase as wild type, but has obviously enhanced extension capacity. The enzyme is used to synthesize long first-strand cDNA and construct overall length of cDNA library at high proportion. The optimal reaction temperature is 42℃.

Components:

M-MLV（RNaseH-）

5 × RT Buffer

Unit Definition:

Unit activity is calculated assuming a specific enzyme activity of 350,000 units per mg protein. Protein is determined by a modification of the Lowry method, using BSA as a standard.

One unit of M-MLV incorporates 1 nmol dTTP into acid-precipitable material in 10 minutes at 37℃， using poly(A) oligo$(dT)_{12\text{-}18}$ as template primer.

Buffer component:

Storage Buffer:	5 × RT Buffer
20mM Tris-HCl (pH7.5)	250mM Tris-HCl(pH8.3)
1mM DTT	375mM KCl
0.01%(v/v) Nonidet-P40	15mM $MgCl_2$
0.1mM Na_2EDTA	50mM DTT
0.1M NaCl	
50%(v/v) glycerol	

Quality Control Assays:

This product has passed the following quality control assays: SDS-polycarylamide get analysis for purity; yield and length of cDNA product; functional absence of DNA endonuclease. Store the 5 × First Strand Buffer at -20℃. Thaw the solutions at room temperature just prior to use and refreeze immediately. The enclosed buffers were assayed with the enzyme and met quality control specifications.

Reaction volume：

components	volume
5×RT Buffer	4μl
10mM dNTPs (10mM)	1μl
Oligod（T）$_{12\text{-}18}$ (10μM)	1μl
mRNA/total RNA	5ng-500ng/50ng-5μg
M-MLV（RNaseH-）（50U/μl）	1μl
DEPC-H_2O	to 20μl

Reaction condition: 42℃, 60min, 95℃, 5min, 4℃, 5min.

cDNA can be stored at -20℃ or used in PCR directly.