AMV Reverse Transcriptase

Description:

AMV Reverse Transcriptase (AMV RT) is isolated from avian myeloblastosis virus. Molecular weight of αβ holoenzyme is 157KD. The enzyme is used to synthesize cDNA and sequencing RNA and DNA. AMV RT catalyzes the polymerization of DNA using total RNA or polyA+ RNA. The enzyme possesses an intrinsic RNase H activity for the degradation of RNA within RNA/DNA heterozygote. Activating agent for AMV RT is Mg2+ or Mn2+ and its optimal reaction temperature is 42℃. The reaction temperature can be 37~41℃ in the case of sodium pyrophosphate. The enzyme is used to synthesize first-strand cDNA up to 10 kb.

Components:

AMV

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)$_{12-18}$ 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$_2$EDTA	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）$_{12-18}$ （10M）	1μl
mRNA/total RNA	5ng-500ng/50ng-5μg
AMV（30U/μl）	1μl
DEPC-H$_2$O	to 20μl

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

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