

AMV Reverse Transcriptase

Applications:

- RT PCR
- Synthesis of cDNA
- RNA Sequencing

Description:

AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrids. The enzyme possesses an intrinsic RNase H activity. AMV RT possesses multiple enzymatic activities including RNA- and DNA-directed DNA polymerase, DNA-RNA unwinding activity, a sequence-specific Mn²⁺-dependent endonuclease and ribonuclease H.

Concentration: 10 u/μl

Storage Buffer:

200mM potassium phosphate (pH7.2), 0.2% Triton X-100, 2mM DTT and 50% glycerol

Reaction Buffer 5X:

250mM Tris-HCl (pH8.3 @ 250 C), 250mM KCl, 50mM MgCl₂, 2.5mM spermidine and 50mM DTT

Unit definition:

One unit is the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C. Reaction conditions are: 50mM Tris-HCl (pH 8.3), 8.75 mM MgCl₂, 40 mM KCl, 10 mM DTT, 0.1 mg/ml BSA, 1 mM radio labelled dTTP and 0.25mM poly(A):oligo(dT).

Quality control:

First-Strand cDNA Synthesis: First strand cDNAs, of 1.2 kb Control RNA is synthesized using 30 units of enzyme, 1 μg of each template, an oligo(dT) primer and a radiolabelled dNTP. The minimum specification is the production of 120 ng of first-strand cDNA. Full-length cDNA must be observed by gel electrophoresis and autoradiography.

Endonuclease Activity: 1 μg of Type I supercoiled plasmid DNA is incubated with 25 units of enzyme in 50mM Tris (pH8.3), 40mM KCl, 7mM MgCl₂, 10mM DTT for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Nuclease Activity: 50 ng of radiolabelled DNA or RNA is incubated with 25 units of enzyme in 4 mM Tris (pH8.3), 3.2 mM KCl, 0.56 mM MgCl₂, 0.8 mM DTT for one hour at 37°C, and the release of radiolabelled nucleotides is monitored by scintillation counting of TCA-soluble material. Passing specifications is <1% release for DNase and <3% release for RNase.

Usage:

Standard Protocol:

We recommend to prepare 2 Mixes

Mix I

Component	Amount/conc.
RNA or polyA RNA	2 μg 50-500 ng
primer	500 ng for each μg of RNA
sterile Water	up to 8 μl (max 11μl)
gently vortex	
Incubation	Temperature
5 min	70 °C
5 min	chill on ice

Mix II

Component	Amount/conc.
AMV 5X reaction buffer	5 µl
dNTP mix (10 mM of each = 40 mM)	2.5 µl
optional: RNAsin	20-40 units
sodium pyrophosphate (40 mM) @ 42°C	2,5 µl (
AMV Reverse (10 u/µl)	3 µl (30 units)
sterile water	up to 25 µl
combine Mix I and Mix II and vortex gently	
prepare a tube containing containing fresh 2–5 µCi [α - ³² P]dCTP	
Transfer 5 µl of the master mix (Mix I and Mix II) to that tube (Mix III)	
Incubate 60 min for Oligo(dT) primers or 60 min for random hexamer primer	42°C 37°C
after incubation: place the samples on ice	
Add 95 µl of 50 mM EDTA to Mix III (can be used for gel analysis)	
Perform second-strand synthesis using the unlabeled first-strand reaction	

Transportation: on blue ice

Storage: at -20°C for 24 months

Ordering Information:

Cat.-no	Description	Amount
105-400	AMV Reverse Transcription	200 units
105-410	AMV Reverse Transcription	5x200 units