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 (pH 8.3 @ 25 °C), 250mM KCl, 50mM $MgCl_2$, 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 $MgCl_2$, 40 mM KCl, 10 mM DTT, 0.1 mg/ml BSA, 1 mM radiolabelled dTTP and 0.25mM poly(A):oligo(dT).

Quality control:
First-Stand 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 370C. 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 $MgCl_2$, 0.8 mM DTT for one hour at 37°C, and the release of radiolabeled 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 gently vortex	up to 8 µl (max 11µl)
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 Reverase (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 Transcriptase	200 units
105-410	AMV Reverse Transcriptase	5x200 units