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AMV Reverse Transcriptase Product No. AMV0500 - 500 U
AMV1000 - 2 x 500 U

AMV Reverse Transcriptase is both an RNA-dependent and DNA-dependent DNA polymerase purified for Avian Myeloblastosis Virus. It is most commonly used for first- and second-strand cDNA synthesis, but can also be used for dideoxy DNA sequencing, RNA sequencing, primer extension, 3'-end labeling of DNA fragments, generation of single-stranded probes for hybridization, and genomic foot printing.

AMV Reverse Transcriptase activity requires either Mg^{2+} or Mn^{2+}, and a primer of greater than eight (8) nucleotides in length.

Concentration: 20 U/µl (500 units/vial)

Storage Buffer: 200 mM potassium phosphate (pH 7.2), 2 mM DTT, 0.2% Triton-X 100, 50% glycerol.

Storage Conditions: For long term storage (> 2 weeks) store aliquots of enzyme at -80°C. The enzyme can be stored at -20°C for no more than 2 weeks. *DO NOT STORE IN A FROST-FREE FREEZER*. Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes.

Physical Purity: Greater than 95% by SDS PAGE. Free of detectable RNase.

Unit Definition: One unit is defined as that amount of enzyme which incorporates one nanomole of dTMP into acid-insoluble product in 10 minutes at 37°C.

Enzyme Assay : Reaction mixtures for the assay consist of 50 mM Tris-HCl, pH 8.3, 6 mM $MgCl_2$, 40 mM KCl, 0.5 mM [^{3}H]dTTP (10-20 cpm/pmol), 0.2 mM polyriboadenylate:deoxythymidylate, and enzyme diluted sufficiently with 10 mM potassium phosphate (pH 7.3) to produce linear incorporation kinetics for at least 30 minutes. The acid-insoluble product of this reaction is collected on 1.5 cm GF/C filter disks and processed for detection of radioactivity by liquid scintillation spectrometry.

Handling of the enzyme should be done with *gloved hands only!* Skin surface is a rich source of non-specific RNases which will render the enzyme useless if contaminated. Care must be taken to ensure against RNase contamination.We suggest not allowing the enzyme to totally thaw during handling. While handling the enzyme (*counting vials or aliquoting*), keep the enzyme on crushed or dry ice. The buffer in which the enzyme is dissolved includes 50% glycerol which will not freeze at -20° C (only -70°C). Keep the vials upright in a rack as much as possible in order to keep the enzyme solution at the bottom of the vial, especially when thawing. It is undesirable for the enzyme to be put through multiple freeze/thaw cycles, BUT in our experience, one or two cycles is not damaging.

If you are switching from RT obtained from another supplier, please be acutely aware of the differences in specific activity and enzyme concentration. Ours is much higher than any other on the market and should be handled as such. You can usually reduce the amount of enzyme needed per reaction (about 5-15 units is an average starting range for ours). The enzyme can be diluted 5 fold.

We recommend Tris-Chloride buffer with our AMV-RT for cDNA reactions. It is critical that the pH is exactly 8.3! After dissolving the enzyme, you must adjust the pH to all components; especially nucleotides to pH of 8.0-8.3-non-acidic.

The optimal temperature for cDNA reactions using AMV-RT is briefly covered in a reference from **Freeman, W.M., Vrana, S.L and Vrana, K.E., Biotechniques,, vol 20, 782-783, 1996.** According to the results, our enzyme can be used optimally for greater sensitivity at a range of 47-55°C. This is contrary to past beliefs that AMV could not be used at high temperatures. Full length cDNA synthesis is easily achieved using these conditions.

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For Research Only