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AB-0321 / v4 / 0607

AMV Reverse Transcriptase

Ordering Information:	AB-0321	AMV Reverse Transcriptase	500 units
	AB-0321/b	AMV Reverse Transcriptase	1,000 units

Enzyme Source: Purified from Avian Myeloblastosis Virus.

Concentration: 21 units/µl

Unit Definition: One unit is the amount of enzyme that incorporates 1nmol of TTP into acid-insoluble form in 10 minutes at 37°C using poly-A-oligo dT_{12-18} as substrate.

Analysis Conditions:

50mM	Tris-HCl, pH 8.3 (at 25°C)
40mM	KCl
6mM	$MgCl_2$
0.5mM	TTP
0.4mM	poly A:oligo dT_{12-18}
2 units	AMV RT

Incubation at 37°C.

Reaction Buffer (10x):

500mM	Tris-HCl, pH 8.3 (at 25°C)
500mM	KCl
100mM	$MgCl_2$
40mM	DTT

Storage and Dilution Buffer:

0.2mM	KH_2PO_4, pH 7.2 (at 25°C)
2mM	DTT
0.2%	Triton® X-100
50% (v/v)	Glycerol

Storage Conditions: Store at -20°C. Shipped on dry ice.

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Exonuclease Activity: Incubation of 50 units of the enzyme with 1µg λ DNA for 16 hours at 37°C in the stated reaction buffer does not produce any detectable degradation of the DNA.

RNase Assay: No detectable RNase activity was observed when 50 units of the enzyme were incubated with 8ng RNA in a 20µl reaction volume for 24 hours at 37°C.

Synthetic Activity: When globulin mRNA is incubated for 5 minutes at 37°C with the enzyme, at least 99% of the cDNA is full length. Using AMV and ^{35}S-mRNA, 44% of the cDNA was 4.4kb.

Applications:

1. Synthesis of cDNA
2. Filling in and labelling the 3'-termini of DNA with overhanging 5' ends
3. DNA sequencing
4. RNA amplification

Protocol: ABgene® recommends using 5 units of AMV per µg of mRNA in a total reaction volume of 25µl. However conditions may vary with different templates

References:

1. Kacian, DL (1977) *Methods Virol* **6**, 143
2. Goodman, HM and MacDonald RJ (1979) *Methods Enzymol* **68**, 75
3. Berger, SL *et al*. (1983) *Biochemistry* **22**, 2365.

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