AMV Reverse Transcriptase

1-800-632-7799
info@neb.com
www.neb.com

M0277S 008120714071

M0277S

200 units **Lot: 0081207** **Exp: 7/14**

10,000 U/ml **Store at –20°C**

Description: Avian Myeloblastosis Virus (AMV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template (1-3).

Source: Avian Myeloblastosis Virus (AMV)

Applications:

- cDNA Synthesis
- RNA Sequencing
- RT-PCR

Supplied in: 0.2 M potassium phosphate, 2 mM dithiothreitol, 0.2% Triton X-100 and 50% glycerol (pH 7.2 @ 25°C).

Storage Note: Once thawed, store at –20°C. Repeated freeze thaw cycle will inactivate the enzyme. Aliquots can be stored for longer periods at –70°C.

Reagents Supplied with Enzyme: 10X AMV Reverse Transcriptase Reaction Buffer.

Reaction Conditions: 1X AMV Reverse Transcriptase Reaction Buffer, supplemented with dNTPs (not included). Incubate at 37–42°C.

1X AMV Reverse Transcriptase Reaction Buffer:
50 mM Tris-HCl
75 mM potassium acetate
8 mM magnesium acetate
10 mM DTT
pH 8.3 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C using poly(rA)•oligo(dT) as template primer.

Unit Assay Conditions: 75 mM potassium acetate, 50 mM Tris-HCl (pH 8.3) 8 mM magnesium acetate, 0.5 mM [^{3}H]-dTTP, 0.2 mM poly(rA)•oligo(dT)12–18 and 10 mM DTT.

Quality Assurance: AMV Reverse Transcriptase is tested for its ability to synthesize full length cDNAs from crude or purified RNA templates. Purified free of detectable levels of RNase, endonuclease and exonuclease activities. AMV Reverse Transcriptase is greater than 95% pure by SDS-Gel Electrophoresis.

Quality Control Assays

DNA Endonuclease Activity: Incubation of a 50 µl reaction containing 30 units of AMV Reverse Transcriptase with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

DNA Exonuclease Activity: Incubation of a 50 µl reaction containing 30 units of AMV Reverse Transcriptase with 1 µg of a mixture single and double-stranded [^{3}H] DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.2% of the radioactivity.

RNase Assay: Incubation of a 10 µl reaction containing 30 units of AMV Reverse Transcriptase with 40 ng RNA transcript for 4 hours at 37°C resulted in no detectable degradation of the RNA as determined by agarose gel electrophoresis.

Note: The yield and size of cDNA transcript increases with increasing amounts of RT.

References:

1. Kacian, D.L. (1977) *Meth. Virol.,* 6, 143.
2. Krug, M.S. et al. (1987) *Meth. Enzymol.*, 152, 316–325.
3. Sambrook J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp 8–64). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

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