



AMV Reverse Transcriptase

F-570
20 U/μl

Store at -70 °C (-20 °C)*

The AMV Reverse Transcriptase is isolated from Avian Myeloblastosis Virus as the $\alpha\beta$ holoenzyme of molecular weight 157,000 daltons, using a modification of the method described by Houts et al. (1979). This preparation is essentially free of nuclease. It is qualified for dideoxy sequencing of DNA and RNA and also for cDNA synthesis using smaller RNA templates. AMV Reverse Transcriptase (AMV RT) is compatible with 4 mM sodium pyrophosphate and placental RNase inhibitor (RNasin) in cDNA reactions. Optimal temperature is 41-45 °C, however, 37-41 °C is preferred if NaPPi is used.

Storage buffer:

0.2 M KPO₄ (pH 7.2)
2.0 mM DTT
0.2 % Triton X-100
50 % Glycerol (v/v)

Activity assay conditions:

50 mM Tris-HCl (pH 8.3 at 25 °C)
6.0 mM MgCl₂
40 mM KCl
4.0 mM DTT
0.5 mM [³H]-TTP
0.4 mM Poly(rA)•(dT)₁₈

AMV RT is supplied with reaction buffer concentrate which is used as 10X or 5X stock depending on the application (see separate application note).

Reaction buffer concentrate:

250 mM Tris-HCl (pH 8.3 at 25°C)
50 mM MgCl₂
500 mM KCl
20 mM DTT

Thaw at 37 °C. Triturate with pipet tip to dissolve, if necessary.

Dilution buffer:

10 mM KPO₄ (pH 7.2)
2.0 mM DTT
0.2 % Triton X-100
10 % Glycerol (v/v)

**Unit definition:**

One unit is defined as the amount of AMV RT required to catalyze the incorporation of one nmol of dTMP into an acid-insoluble product in 10 minutes at 37 °C using Poly(rA)•(dT)₁₈ as a template primer.

Ribonuclease Assay:

This enzyme is free of exogenous RNase. Incubation of 15 U of AMV RT with 1 µg MS2 RNA (1h, 37 °C, 50 µl) results in the same RNA band as that produced without the enzyme

Exonuclease Activity:

Incubation of 15 U for 4 hours at 37 °C in 50 µl assay buffer with 1 µg sonicated ³H DNA (2x10⁵ cpm/µg) released < 2 % of radioactivity.

Endonuclease Assay:

No endonuclease activity is observed after incubation of 15 U of AMV RT with 1 µg of λ-*Hind*III DNA fragments in assay buffer at 37°C for 4 hours.

Note

Reverse Transcriptase isolated from AMV has an associated Ribonuclease H activity independent of cDNA activity. This endogenous RNase acts as an exonuclease that requires free ends and acts on the RNA moiety of DNA-RNA hybrid.

Reference

Houts, G.E., Miyagi, M., Ellis, C., Beard, D., and Beard, J.W. (1979) *J. Virol.* 29 (2): 517-522.

*Upon receipt, centrifuge the vial in a microfuge to assure the full recovery of the enzyme. In order to conserve optimal activity, it is strongly suggested that the enzyme be aliquoted and stored at -70 °C, although storage at -20 °C is adequate for short periods of time. Repeated freezing and thawing results in loss of enzyme activity. **Do not store this enzyme in a frost-free freezer.** Due to the viscosity of the enzyme, maximum dispensing efficiency is achieved by the use of positive displacement pipets. Stable for one year from the assay date.



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- That this remedy is in lieu of all other remedies or claims for damages, consequential or otherwise, which the buyer may have against Finnzymes Oy.

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