

**CERTIFICATE OF ANALYSIS**

## AMV Reverse Transcriptase

**#EP0641**      1000 u

**Lot:**                      **Expiry Date:**

Concentration:      20 u/μl

Supplied with:      1 ml of 5X AMV RT Buffer

**Store at -20°C**

In total 2 vials.

### Description

AMV Reverse Transcriptase is a recombinant Avian Myeloblastosis Virus reverse transcriptase (AMV RT) expressed in *E.coli*. It is a heterodimer composed of nonidentical subunits  $\alpha$  and  $\beta$ . AMV RT possesses multiple enzymatic activities including RNA- and DNA-directed DNA polymerase, DNA-RNA unwinding activity, a sequence-specific  $Mn^{2+}$ -dependent DNA endonuclease and ribonuclease H.

### Source

*E.coli* cells with a cloned *pol* gene of AMV.

### Features

- Temperature optimum of activity at 45-50°C.
- Highly thermostable, can be used at temperatures up to 60°C.
- Suitable for synthesis of full-length cDNA as long as 13 kb.
- Incorporates modified nucleotides.

### Applications

- First strand cDNA synthesis (1), see protocol on back page.
- RT-PCR and real-time RT-PCR.
- Synthesis of cDNA for cloning and expression.
- RT-PCR of GC-rich templates and templates.
- Synthesis of labeled cDNA probes.
- Analysis of RNA by primer extension (1).

## Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of dTMP into a polynucleotide fraction (adsorbed on DE-81) in 10 min at 37°C.

Enzyme activity is assayed in the following mixture: 50 mM Tris-HCl (pH 8.5), 8 mM MgCl<sub>2</sub>, 30 mM KCl, 1 mM DTT, 0.5 mM dTTP, 0.4 MBq/ml [<sup>3</sup>H]-dTTP, 0.4 mM polyA·oligo(dT)<sub>12-18</sub>.

## Storage Buffer

The enzyme is supplied in: 200 mM potassium phosphate, 2 mM DTT, 0.2% (v/v) Triton X-100, 50% (v/v) glycerol (pH 7.2).

## 5X AMV RT Buffer

250 mM Tris-HCl (pH 8.5 at 25°C), 40 mM MgCl<sub>2</sub>, 150 mM KCl, 5 mM DTT.

## Inhibition and Inactivation

- Inhibitors: metal chelators, inorganic phosphate. Pyrophosphate (Na-PP<sub>i</sub>) at a 0.5 mM concentration reduces AMV RT activity to 50%. Na-PP<sub>i</sub> at a ≤4 mM concentration increases the yield of full-length cDNA significantly (2).
- Inactivated by heating at 85°C for 5 min.

## QUALITY CONTROL ASSAY DATA

### Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25 units of the enzyme with 1 µg of pUC19 DNA in 50 µl of reaction buffer for 16 hours at 37°C.

### Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 40 units of the enzyme with 1 µg of sonicated *E. coli* [<sup>3</sup>H]-DNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 40 units of the enzyme with 1 µg of [<sup>3</sup>H]-RNA in 50 µl of reaction buffer for 4 hours at 37°C.

### Functional Assay

AMV Reverse Transcriptase was functionally tested for use in the first strand cDNA synthesis.

Quality authorized by:



Jurgita Zilinskiene

(continued on back page)

## Protocol for First Strand cDNA Synthesis

The following protocol is optimized to generate first-strand cDNA for use in two-step RT-PCR.

Mix and briefly centrifuge all components after thawing, keep on ice.

- ❶ Add into sterile, nuclease-free tube on ice in the indicated order:

total RNA <i>or</i> poly(A) <sup>+</sup> mRNA	10 ng-5 µg  1-100 ng
Oligo(dT) <sub>18</sub> primer, 0.5 µg/µl (#SO131) <i>or</i> Random hexamer primer, 0.2 µg/µl (#SO141) <i>or</i> gene-specific primer	1 µl (0.5 µg)  1 µl (0.2 µg)  15-20 pmol
DEPC-treated water (#R0601)	to 13 µl

- ❷ **Optional:** If RNA template is GC rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65°C for 5 min, chill on ice, briefly centrifuge and place on ice.

- ❸ Add the following components in the indicated order:

5X AMV RT buffer	4 µl
RiboLock™ RNase Inhibitor (#EO0381)	0.5 µl (20 u)
dNTP Mix, 10 mM each (#R0191)	2 µl (1 mM final concentration)
AMV Reverse Transcriptase	0.5 µl (10 u)
<b>Total volume</b>	<b>20 µl</b>

Mix gently and centrifuge to collect all drops.

- ❹ If oligo(dT)<sub>18</sub> primer or gene-specific primer is used, incubate 60 min at 50°C.  
If random hexamer primer is used, incubate 10 min at 25°C followed by 60 min at 50°C.  
For transcription of GC rich RNA reaction temperature can be increased to 60°C.
- ❺ Terminate the reaction by heating at 85°C for 5 min. Do not heat-inactivate enzyme prior to analysis of long cDNA to avoid cleavage.

### Note

- The reverse transcription reaction product can be directly used in PCR or stored at -20°C.
- Use 2 µl of the reaction mix to perform PCR in 50 µl volume.

## Reference

1. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
2. Eun, H.-M., Enzymology Primer for Recombinant DNA Technology, Academic Press, Inc., 1996.

**DE-81** – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.

**Whatman** is a registered trademark of Whatman Ltd.

**Triton X-100** is a registered trademark of Rohm & Haas, Inc.

### **PRODUCT USE LIMITATION.**

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to [www.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.

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## Related Products

- Oligo(dT)<sub>18</sub> Primer #S0131
- Random Hexamer Primer #S0141
- dNTP Mix, 10 mM each #R0191
- dNTP Mix, 25 mM each #R1121
- Aminoallyl-dUTP #R0091
- Biotin-11-dUTP #R0081
- Fluorescein-12-dUTP\* #R0101
- RiboLock™ RNase Inhibitor #E00381
- DreamTaq™ DNA Polymerase #EP0701
- Taq DNA Polymerase (recombinant) #EP0401
- Pfu DNA polymerase (native)\* #EP0571
- Pfu DNA polymerase (recombinant)\* #EP0501
- PCR Master Mix (2X) #K0171
- Maxima™ Hot Start Taq DNA Polymerase #EP0601
- TrueStart™ Taq DNA Polymerase #EP0611
- PyroStart™ Fast PCR Master Mix (2X) #K0211
- High Fidelity PCR Enzyme Mix\* #K0191
- Long PCR Enzyme Mix\* #K0181
- DNase I, RNase-free #EN0521
- RNase H, *E.coli* #EN0201
- DNA Polymerase I, *E.coli* #EP0041
- DEPC-treated Water #R0601
- Water, nuclease-free #R0581

\* not available in the USA