



## **The ENZYME Company**

### **AMV Reverse Transcriptase**

**Cat.-No.: 105410**

**1000 units**

#### **Description**

AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrids (1). It requires a primer (DNA primers are more efficient than RNA primers) as well as  $Mg^{2+}$  or  $Mn^{2+}$ . The enzyme possesses an intrinsic RNase H activity. Please refer to the **Usage Notes**, before using this enzyme.

#### **Applications of AMV RT include:**

- First-strand synthesis of cDNA from RNA molecules (2).
- Sequencing of RNA transcripts (3).

**Concentration:** 5000 – 15.000 units/ml

**AMV Reverse Transcriptase 5X Reaction Buffer:** 250 mM TrisHCl, (pH 8,3 at 25°C), 250mM KCl, 50 mM  $MgCl_2$ , 2,5 mM spermidine and 50 mM DTT.

**Enzyme Storage Buffer:** AMV Reverse Transcriptase (AMV-RT) is supplied in 200 mM potassium phosphate (pH7.2 at 4°C), 0,2 % Triton X-100, 2 mM DTT and 50% glycerol.

**Source:** Purified from avian myeloblastosis virus particles.

**Unit definition** One unit is defined as the amount of enzyme required to catalyze the transfer of 1 mmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C. The reaction conditions are: 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 8.75 mM  $MgCl_2$ , 10 mM DTT, 0,1 mg/ml acetylated BSA, 1 mM radiolabeled dTTP and 0.25 mM poly(A): oligo(dT). See the unit concentration on the Product Information Label.

**Storage condition:** -20°C

#### **First-Strand Synthesis of cDNA Protocol**

**1. Mix in the tube:**

2µg of the total RNA (or 50-500ng of polyA RNA)  
500ng of primer for each µg of RNA  
add water up to 8 µl (<11 µl)

**2. Incubate the mixture:** 5 min at 70°C, then chill for 5min on ice

**3. Add into the mixture:**

- 5 µl of **5xRT buffer complete** (250mM TrisHCl, pH8,3; 500mM KCl, 15mM  $MgCl_2$ , 50mM DTT)
- 2,5 µl of dNTP mix (10mM of each dNTP; Cat.-No: 110001 and 110002)
- RNAsin 40 units (optional)
- 2,5 µl sodium pyrophosphate, 40mM (prewarmed to 42°C)
- AMV RT 30 units
- $H_2O$  (nuclease-free) – up to 25 µl

**4. Mix gently:** transfer 5 µl of the reaction mixture to another tube containing 2-5 µCi [ $\alpha$ - $^{32}P$ ]dCTP. Do not add lable to the remaining 20µl reaction. **Note:** We recommend using [ $\alpha$ - $^{32}P$ ]dCTP that is less than 1 week old.

**5. Incubate the mixture:** for 60 min at 42°C for oligo(dT) primers or at 37°C for random hexamer primers.

**6. Enzyme inactivation:** Place the reactions, labelled and unlabeled, on ice and add 95µl of 50 mM EDTA to the labelled (tracer) reaction. The reaction volume should now total 100 µl. The Tracer reaction may be used for an incorporation assay and gel analysis.

**7. Perform second-strand synthesis:** using the unlabeled first-strand reaction see reference (4). No phenol extraction or ethanol precipitation is necessary.

#### **Sequencing of RNA Transcripts**

A protocol for sequencing RNA transcripts may be found in reference (3).

#### **Usage Notes:**

1. The formulation of AMV Reverse Transcriptase 5x Reaction buffer is **not** compatible with M-MLV Reverse Transcriptase.
2. Up to 10µl of an RT reaction containing AMV RT and supplied AMV Reverse Transcriptase Reaction Buffer can be added to PCR amplification reactions that use Taq DNA Polymerase (Cat.-No.) or PCR Mastermix (Cat.-No.) are used, up to 25µl of the RT reaction can be added to a 50µl PCR.

#### **References**

- (1) Kacian, D.L. (1977) **Methods for Assaying reverse transcriptase. Meth. Virol. 6, 143.**

#### **BIORON International**

Contact Germany Phone: +49 621 5720 915  
Contact Singapore Phone : +65 6896 8063  
Contact Bioron Poland. +48 4267 7045 7  
E-Mail: info@bioron.net WEB: www.bioron.de



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- (2) Krug, M.S. and Berger, S.L. (1987) First-strand cDNA synthesis primed with oligo(dT). *Meth. Enzymol.* 152, 316-25
- (3) Mierendorf, R.C. and Pfeiffer, D. (1987) Sequencing of RNA transcripts synthesized in vitro from plasmids containing bacteriophage promoters. *Meth. Enzymol.* 152, 563-6.
- (4) Sambrook, J. Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 864

Reverse and RTx5 buffer are free from RNase activities, meanwhile we recommend to add RNasin into the mixture to inhibit possible RNase contaminations of the sample.

Catalog #	Pack size
105400	200 units
105410	1000 units

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Contact Germany Phone: +49 621 5720 915  
Contact Singapore Phone : +65 6896 8063  
Contact Bioron Poland. +48 4267 7045 7  
E-Mail: [info@bioron.net](mailto:info@bioron.net) WEB: [www.bioron.de](http://www.bioron.de)