

# DATA SHEET

Version: 03  
Revision date: 05/06/2023

## 1. Identification

<b>Product name</b>	<b>AMV Reverse Transcriptase</b> 1000 U (10 U/ $\mu$ L)
<b>Cat. No</b>	<b>P0071</b>

## 2. Description

**AMV Reverse Transcriptase**, encoded by Avian Myeloblastosis Virus (AMLV) is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template.

AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrids. The enzyme possesses an intrinsic RNase H activity. AMV RT possesses multiple enzymatic activities including RNA- and DNA-directed DNA polymerase, DNA-RNA unwinding activity, a sequence-specific Mn<sup>2+</sup>-dependent endonuclease and ribonuclease H.

**Source:** purified from *E.coli* strain harboring a plasmid that directs the synthesis of modified form of AMLV-RT.

## 3. Protein information

<b>Purity</b>	Purity is >95% by SDS-PAGE
<b>Storage buffer</b>	200mM Potassium phosphate (pH 7.2), 2mM DTT, 0,2% Triton X-100 and 50% glycerol
<b>Reaction Buffer (5X)</b>	250mM Tris-HCl, pH 8.3, 250mM KCl, 50mM MgCl <sub>2</sub> , 50mM DTT
<b>Biological activity</b>	One unit is the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C, using poly(A) oligo dT as a template primer.

## 4. Storage specifications

Store at -20°C. Avoid exposure to constant temperature changes.

## 5. Applications

- RT PCR.
- Synthesis of cDNA.
- RNA Sequencing.

## 6. Further information

<b>Product Use Limitations</b>	This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
<b>Disclaimer</b>	The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.



## DATA SHEET

Version: 03  
Revision date: 05/06/2023

### PROTOCOL

We recommend to prepare 2 Mixes:

#### Mix 1

**1. Mix in the tube:**

Add 2 µg of total RNA (or 50 – 500 ng of poly(A)-RNA) to reverse DNA primer. Use 0.5µg primer/µg RNA in a total volume of 11µl in water. Mix gently by vortex.

**2. Heat the RNA-Mix:**

Incubate the mixture at 70°C for 5 minutes and chill on ice.

#### Mix 2

**3. Mix in the tube:**

- ✓ 5 µl of 5X RT buffer.
- ✓ 2.5 µl of dNTPs mix (10 mM each).
- ✓ [Optional] 20–40 Units RNase inhibitor (Not provided).
- ✓ 2.5 µl 40 mM Sodium Pyrophosphate (prewarmed to 42°C).
- ✓ 3µl of AMV Reverse Transcriptase (10U/ µl).
- ✓ Nuclease-Free Water to final volume 25µl

**4. Combine **Mix1** and **Mix 2** and vortex gently.**

**5. Incubate the reaction:**

Incubate for 60 minutes at 42°C for oligo(dT) primers or at 37°C for random hexamer primers.

**6. Stop the reaction by heating at 70°C for 10 minutes. Chill on ice.**

**7. Use the mixture for the desired application.**

