

Data sheet

AMV Reverse Transcriptase

Cat. No: P0070 (300 U)

Cat. No: P0071 (1,000 U)

Introduction:

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^{2+} -dependent endonuclease and ribonuclease H.

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

Application:

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

Kit Contents

AMV Reverse Transcriptase (10U/ μ L)

Reaction Buffer 5X

Storage:

Store at $-20^{\circ}C$.

Unit definition:

One unit is the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at $37^{\circ}C$, using poly(A) oligo dT as a template primer.

Quality control:

MMLV RT is free of detectable RNase, and DNase (exo- and endonuclease) activities.

Purity: >90% as judged by SDS-polyacrylamide gels with blue staining.

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Protocol

1. Mix in the tube:

Add 2 µg of total RNA (or 50 – 500 ng of poly(A)-RNA) to 1 µl of 10 µM reverse DNA primer and 4 µl of dNTPs mix (5 mM each) in a total volume of 14 µl.

2. Heat the RNA-Mix:

5 min to 65°C and chill on ice for another 5 min.

3. Prepare RT-Mix:

4 µl of 5x RT buffer (250 mM Tris acetate (pH 8.4), 375 mM potassium acetate, 40 mM magnesium acetate and stabilizers), 0.5 µl RNase inhibitor 30 U/µl (Not provided), 1 µl 100 mM DTT and 0.5 µl of AMV Reverse Transcriptase.

4. Add 6 µl of RT-Mix to 14 µl RNA-Mix:

Final volume is 20µl.

5. Incubate the reaction:

15 min at 42°C followed by 45 min at 50°C (or alternatively between 42°C and 65°C).

6. Use the mixture for the desired application.

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.canvaxbiotech.com for Material Safety Data Sheet of the product.

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