For life science research only.
Not for use in diagnostic procedures.

Reverse Transcriptase, AMV
from avian myeloblastosis virus

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Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase (RNA-directed)

Cat. No. 10 109 118 001	1,000 U > 20 U/µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Reverse Transcriptase, AMV	Storage buffer: 200 mM potassium phosphate, 2 mM dithiothreitol, 0.2% Triton X-100 (v/v), 50% glycerol (v/v), pH approximately 7.2.	1 vial, 1,000 U
2	Reverse Transcriptase, AMV, Incubation buffer for AMV Reverse Transcriptase, 5x conc.	▪ 250 mM Tris-HCl, 150 mM KCl, 40 mM $MgCl_2$, 5 mM dithiothreitol, pH 8.5 (+20°C). ▪ For 1st strand cDNA synthesis.	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Reverse Transcriptase, AMV	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	Incubation buffer for AMV Reverse Transcriptase, 5x conc.	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free pipette tips
- 1.5 ml RNase-free microcentrifuge tubes
- Microcentrifuge

For 1st strand cDNA synthesis

- Water, PCR Grade*
- Poly(A)+ RNA
- Poly(A) × $(dT)_{15}$* primer
- Protector RNase Inhibitor*
- PCR nucleotide, such as the PCR Nucleotide Mix*, PCR Nucleotide Mix^{PLUS}*,or the Deoxynucleoside Triphosphate Set*
- [α-^{32}P]dCTP (optional)

1.4. Application

AMV Reverse Transcriptase is used in the following applications:

- cDNA synthesis
- Synthesis of first-strand cDNA for use in subsequent amplification reactions.
- Dideoxy DNA sequencing
- RNA sequencing
- 3′-end labeling of DNA fragments.
- Generation of single-strand probes for genomic footprints.

2. How to Use this Product

2.1. Before you Begin

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

2.2. Protocols

Standard assay for 1st strand cDNA synthesis

1. To set up a 20 µl reaction, pipette the following components into a microfuge tube on ice and mix.

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to a final volume of 20	–
Incubation buffer, 5x conc. (Vial 2)	4	1x
Poly(A)+ RNA	X	2 µg
Poly(A) × $(dT)_{15}$* primer	X	40 mA_{260} U
[α-^{32}P]dCTP (optional)	X	20 µCi (3,000 Ci/mMol = 110 TBq/mMol)
dATP*, dCTP*, dGTP*, and dTTP*	X	20 nMol each
RNase inhibitor*	X	25 U
Reverse Transcriptase, AMV (Vial 1)	X	40 U
Final Volume	**20 µl**	

2. Incubate at +42°C for 60 minutes.

3. The resulting first strand cDNA can be easily used for second strand synthesis, hybridization, or amplification by PCR.
– After the incubation, incorporation rates are >2 × 10^5 cpm (Cerenkov). This corresponds to 15 to 30% conversion to cDNA, and depends on the quality of the template RNA. The obtained transcripts are >90% full length.

i A protocol for the preparation of 2nd strand cDNA can be found in the Instructions for Use of the cDNA Synthesis System.*

2.3. Parameters

EC-Number

EC 2.7.7.49

Molecular Weight

68 kD (α-subunit); 92 kD (β-subunit)

Specific Activity

>50 U/µg

Unit Definition

1 unit is the enzyme activity that incorporates 1 nmol of [^{3}H]TMP into acid-insoluble products in 10 minutes at +37°C with poly(A) × $(dT)_{15}$ as substrate.

Volume Activity

>20 U/µl

3. Additional Information on this Product

3.1. Test Principle

Reverse Transcriptase, AMV is a gene product of the RNA genome of avian myeloblastosis virus. The enzymatically active forms of the purified enzyme are α, ββ, and αβ. The mature αβ form, the most active form of AMV reverse transcriptase, includes an RNA-directed DNA polymerase, DNA-dependent DNA polymerase, RNase H, and an unwinding activity.

3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
PCR Nucleotide Mix^{PLUS}	2 x 100 µl, 200 PCR reactions in 50 µl	11 888 412 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Poly(A) x $(dT)_{15}$	5 U, A_{260} units	10 108 677 001
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 622 614 001
cDNA Synthesis System	1 kit, up to 10 reactions	11 117 831 001
Protector RNase Inhibitor	2,000 U, 40 U/µl	03 335 399 001
	10,000 U, 5 x 2,000 U	03 335 402 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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