3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

# ProductInformation

**Enhanced Avian Reverse Transcriptase**

Product Number. **A 4464**
Storage Temperature –20 °C

## Product Description

Sigma's Enhanced Avian Reverse Transcriptase is a highly purified, avian myeloblastosis virus reverse transcriptase (AMV-RT) that offers superior performance to standard AMV-RT or standard Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT). This exceptionally robust AMV-RT has an enhanced ability to transcribe through difficult secondary structure at elevated temperatures (up to 65 °C)[1,2] making it the ideal enzyme for producing high quality full-length cDNA from total RNA or poly(A)$^+$ RNA with difficult secondary structure. This means greater full-length cDNA synthesis and higher yields when transcribing total RNA or poly(A)$^+$ RNA templates with difficult secondary structure.[1,2] eAMV-RT is also very efficient at transcribing long targets.[3]

Enhanced Avian RT Unit Definition: One unit incorporates one nanomole of TMP into TCA precipitable material in 10 minutes using polyadenylic acid as template and oligo $(dT)_{12-18}$ as a primer.

## Reagents Provided

- Enhanced Avian Reverse Transcriptase, Product No. A 4714, 200 mM $KH_2PO_4$ , pH 7.2, 2 mM DTT, 0.2% Triton, 50% glycerol, 20 units/µl — 500 units/1,000 units
- 10X Buffer for AMV Reverse Transcriptase, Product No. B 1175, 500 mM Tris-HCl, pH 8.3, 400 mM KCl, 80 mM $MgCl_2$, 10 mM DTT — 1.5 ml

## Reagents Required but not Provided

- RNA to be transcribed
- Random nonamer primer (Product No. R 7647), Anchored oligo $(dT)_{23}$ (Product No. O 4387) or specific primer for RT
- Deoxynucleotide Mix, Product No. D 7295, 10 mM dATP, 10 mM dCTP, 10 mM dTTP, 10 mM dGTP
- RNase Inhibitor, Product No. R 2520
- Water, PCR Reagent, Product No. W 1754
- Dedicated pipets
- Aerosol resistant pipet tips
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Product No. P 3114 and P 3364

## Precautions and Disclaimer

Sigma's Enhanced Avian Reverse Transcriptase Set is for R&D use only. Not for drug, household or other uses. Warning statements are included on the label or in the Reagents Provided section of this bulletin where applicable. In addition, when using radioactively labeled nucleic acids, standard procedures for safely handling radioactive materials should be followed.

## Storage

All components should be stored at –20 °C.

## Preliminary Considerations

RNA Preparation

The single most important step in assuring success is high quality RNA preparation. Integrity and purity of RNA template is essential. Either total or poly(A)$^+$ RNA can be used as template for the reverse transcription reaction. All RNA preparations should be DNA-free to assure that product is derived from RNA. DNase 1 (Product Code: AMP-D1) is recommended for the digestion of contaminating DNA in the RNA preparation before the first strand synthesis reaction. The minimum amount of RNA that can be amplified is both primer and template dependent. For total RNA or poly(A)$^+$ RNA, amplified product is obtained using as little as 10-100 pg of starting material, depending on number of RNA copies present.

Primer Design

Specific primers for reverse transcription should be designed with the aid of primer design software to eliminate the complications introduced with primer-dimers and secondary structures. In addition, selection of primers that span an intron will greatly reduce the possibility of amplifying from the genomic DNA. This will also allow genomic amplification products to be identified by their larger size. Random nonamers and anchored oligo $(dT)_{23}$ (as provided in the First Strand Synthesis Kit, STR-1 or as individual products) are alternatives to specific primers for first strand synthesis, cDNA library construction and other applications.

**Procedure**
The optimal conditions for the concentration of Enhanced Avian Reverse Transcriptase, template RNA, and primers will depend on the system being utilized and should be determined empirically.

1. Add the following reagents to a thin-walled 200 µl or 500 µl PCR microcentrifuge tube on ice:

| Volume | Reagent | Final Concentration |
|---|---|---|
| - µl | RNA Template (0.1-5 µg total RNA or desired amount of poly(A)$^+$ RNA) | 0.005-0.25 µg/µl total RNA or desired amount of poly(A)$^+$ RNA) |
| 1 µl | Deoxynucleotide Mix | 500 µM each dNTP |
| 1 µl | 3' Antisense specific primer<br>-or-<br>Random nonamers<br>-or-<br>Anchored oligo $(dT)_{23}$ | 1 µM (In general, use between 0.5-1 µM)<br><br>2.5 µM (In general, use between 1-4 µM)<br><br>3.5 µM (In general, use between 1-3.5 µM) |
| - µl | Water, PCR Reagent | ------- |

10 µl Total Volume

2. Mix gently and briefly centrifuge to collect all components to the bottom of the tube.

3. Place tube in the thermal cycler at 70 °C for 10 minutes.

Note: This 70 °C incubation step before the reverse transcription reaction is optional. This step may denature RNA secondary structure, which will allow for more efficient reverse transcription. All of the reverse transcription components may be added together in one tube and placed immediately at the optimal reverse transcription temperature unless random primers are being used which would require a 15 minute incubation at 25 °C before the optimal RT temperature incubation.

4. Remove tube, place on ice, centrifuge and add the following components to the reaction:

| Volume | Reagent | Final Concentration |
|---|---|---|
| 2 µl | 10X Buffer for AMV-RT | 1X |
| 1 µl | Enhanced AMV-RT | 1 U/µl |
| 1 µl | RNase Inhibitor diluted to 20 units/µl | 1 U/µl |
| 6 µl | Water, PCR Reagent | |

20 µl Total Volume

Note: Increased yield for longer templates may be obtained by increasing the concentration of deoxynucleotides from 500 µM each dNTP to 1 mM each dNTP.

5. Incubate the reaction tubes at 25 °C for 15 minutes if using random primers. If using oligo $(dT)_{23}$ or a specific primer, this step is not needed. This preincubation step allows these primers to be extended by the eAMV reverse transcriptase before incubating at a temperature between 42-50 °C.

6. Place tubes at a temperature between 42-50 °C for 50 minutes.

Note: The optimal reaction temperature should be determined empirically. Raising the transcription reaction temperature incrementally (up to 65 °C) is recommended for transcribing templates with difficult secondary structure. If the transcription reaction is run at elevated temperatures, a drop in yield may occur.

7. The first strand cDNA is now ready for subsequent PCR amplification.

**References**

1. Eastlund, E., and Song, K., Sigma's New Enhanced Avian RT-PCR Kit. Sigma-Aldrich Corporation's Life Science Quarterly, **1,** 15-17, (2000).
2. Brooks, E.M., et al., Secondary structure in the 3' UTR of EGF and the choice of reverse transcriptases affect the detection of message diversity by RT-PCR. Biotechniques **19,** 806-812 (1995).
3. Eastlund, E., and Mueller, E., Hot Start RT-PCR Results in Improved Performance of the Enhanced Avian RT-PCR. Sigma-Aldrich Corporation's Life Science Quarterly, **2,** 2-5, (2001).

**Related Products**

RNA Isolation

GenElute™ Mammalian Total RNA Purification Kit, for isolating total RNA from tissue or cells, Product Codes RTN10, RTN70 and RTN350

GenElute Direct mRNA Miniprep Kit, for isolating mRNA from cells or tissue, Product Codes DMN10 and DMN70

GenElute mRNA Miniprep Kit, for isolating mRNA from total RNA, Product Codes MRN10 and MRN70

TRI Reagent®, for isolating total RNA from Tissue Product Code T 9424

TRI Reagent® BD, for isolating total RNA from whole blood, Product Code T 3809

TRI Reagent® LS, for isolating total RNA from fluid samples, Product Code T 3934

RNaseZAP®, a cleaning product for removing RNase from laboratory surfaces, Product Code R 2020

Deoxyribonuclease I, amplification grade, for removing DNA from RNA preps, Product Code AMP-DI

RNA*later*™ for long-term RNA storage, Product Code R 0901

PCR Products

JumpStart™ AccuTaq™ LA DNA Polymerase Mix, Product Code D 5809

PCR Optimization Kit II, Product Code OPT-2

RT-PCR Products

Enhanced Avian RT-PCR Kits for both one-step and two-step RT-PCR reactions, Product Codes HS-RT100 (100 reactions) and HS-RT20 (20 reactions)

PCR Literature

*PCR: Essential Data Series*, Newton, C. R. (Ed.), (John Wiley and Sons, Inc.,New York, NY, 1995). Product Code Z36,491-6

*PCR Primer: A Laboratory Manual*, Dieffenbach, C., and Dveksle, G. S.,(Eds.) (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1995). Product Code Z36,411-8

*PCR Protocols: A Guide to Methods and Applications*, Innis, M. A., et al., (Eds.) (Academic Press, San Diego, CA, 1990). Product Code P 8177

*PCR Sequencing Protocols*, Rapley, R., (Humana Press, Totowa, NJ, 1996). Product Code Z37,381-8

*PCR Strategies*, Innis, M. A., et al., (Eds.) (Academic Press, San Diego, CA, 1995). Product Code Z36,445-2

*Quantitation of mRNA by Polymerase Chain Reaction*, Kohler, T., et al., (Springer-Verlag, Berlin, 1995). Product Code Z37,194-7

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.



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