RT-PCR Product Listing

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eAMV™ Reverse Transcriptase

from Avian Myeloblastosis Virus

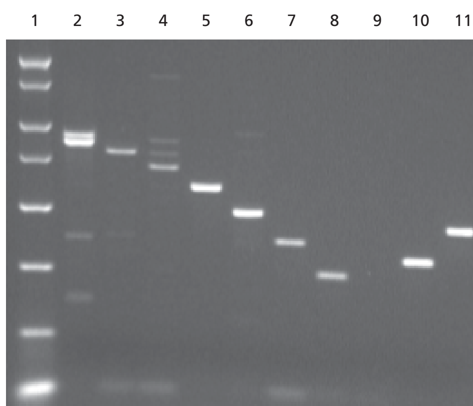
eAMV Reverse Transcriptase is an enhanced form of Avian Myeloblastosis Virus (AMV) RT that synthesizes a DNA strand complementary to RNA, DNA or an RNA:DNA hybrid.

This exceptionally robust AMV RT has greater thermostability than standard AMV or M-MLV reverse transcriptase. eAMV exhibits an enhanced ability to detect low abundance messages, transcribe through difficult secondary structure and transcribe mRNA templates up to 14.1 kb.

Features and Benefits

- Produces first strand cDNA ready for PCR amplification
- Produces full-length cDNA, up to 14.1 kb, with higher yields
- More efficient at transcribing through difficult secondary structure than AMV-RT, M-MLV RT or RNase H-minus M-MLV RT
- Detects low abundance mRNA better than RNase H-reduced AMV-RT or RNase H-minus M-MLV RT

RNA Reverse Transcribed up to 14 kb in Length



Components: eAMV Reverse Transcriptase
10× Buffer for eAMV Reverse Transcriptase

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

Concentration: 20 units per µl

Storage: –20 °C
Shipped in wet ice

References

1. 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).
2. Tosh, C., et al., One-tube and one-buffer system of RT-PCR amplification of 1D gene of foot-and-mouth disease virus field isolates, *Acta Virol.* **41,** 153-155 (1997).
3. Dukas, K.P., et al., Quantitation of changes in the expression of multiple genes by simultaneous polymerase chain reaction, *Analyt. Biochem.* **215,** 66-72 (1993).

Ordering Information

Cat. No.	Product Description	Quantity
A4464	eAMV Reverse Transcriptase	500 units 1,000 units

RNA reverse transcribed up to 14 kb in length. mRNA from HEK293 cells was reverse transcribed to a length of 14.1 kb using eAMV Reverse Transcriptase. The resulting cDNA was amplified using JumpStart REDTaq with PCR primer sets chosen at different distances from the poly (A)$^+$ tail resulting in the following PCR products. Successful amplifications demonstrate the integrity of the cDNA up to 14 kb.

Lanes 2-8 are primer sets for p619, Lane 10 is β-actin and Lane 11 is GAPDH.

Lane 1: Marker
Lane 2: 908 bp PCR product, 2,180 transcript size
Lane 3: 796 bp PCR product, 5,760 transcript size
Lane 4: 708 bp PCR product, 6,875 transcript size
Lane 5: 608 bp PCR product, 9,970 transcript size
Lane 6: 499 bp PCR product, 10,980 transcript size
Lane 7: 396 bp PCR product, 12,890 transcript size
Lane 8: 300 bp PCR product, 14,150 transcript size
Lane 9: Negative Control (No RT)
Lane 10: 349 bp PCR product, 1,060 transcript size
Lane 11: 452 bp PCR product, 880 transcript size

eAMV™ First Strand Synthesis Kit

Going to greater lengths—up to 14.1 kb cDNA transcripts

The eAMV First Strand Synthesis Kit is the ideal way to generate stable complementary DNA (cDNA) from fragile total RNA or poly(A)+ RNA. eAMV will effectively transcribe messages, even ones that are of very low abundance. It is more stable at elevated temperatures, up to 65 °C, which allows it to transcribe through difficult secondary structure better than other commercially available reverse transcriptases. With this kit, a dependable cDNA is generated that can be used for various downstream applications, including PCR.

Unit definition: One unit incorporates one nanomole of TMP into TCA-precipitable material in 10 min using polyadenylic acid as template and oligo$(dT)_{12\text{-}18}$ as primer

Components: eAMV Reverse Transcriptase, 1,000 units
10× Buffer for eAMV Reverse Transcriptase, 1.5 ml
Deoxynucleotide mix, 50 µl
Anchored oligo $(dT)_{23}$, 100 µl
Random nonamers, 100 µl
RNase Inhibitor, 1 vial
PCR Grade Water, 1.5 ml

Storage: –20 °C
Shipped in dry ice

Reference

1. 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).

Ordering Information

Cat. No.	Product Description	Quantity
STR1	eAMV First Strand Synthesis Kit Sufficient for 50 reactions	1 kit

RT-PCR

eAMV™ HS RT-PCR Kits

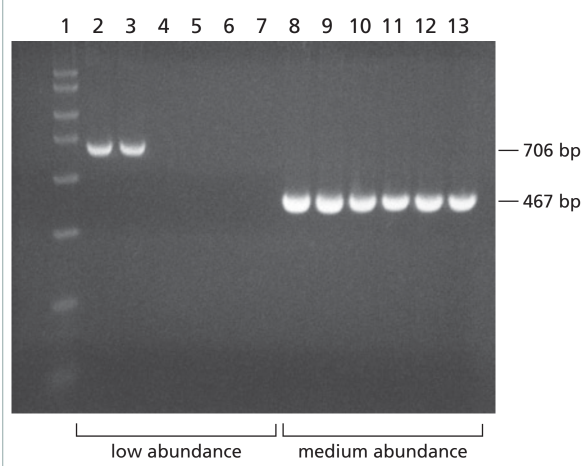
The best choice for amplification of difficult templates and low abundance messages

Reverse Transcription PCR (RT-PCR) is a powerful tool used to study gene expression. The eAMV RT-PCR Kit provides a fast, convenient and reliable system with a number of advantages. eAMV Reverse Transcriptase is able to detect low abundance messages, transcribe through difficult secondary structure and generate cDNA templates up to 14.1 kb. Its broad range of thermal activity helps disrupt secondary structures and makes eAMV the most robust reverse transcriptase at temperatures up to 65 °C. This kit includes JumpStart AccuTaq LA DNA PCR Polymerase which increases specificity and sensitivity of the amplified product. The combination of these two enzymes into one RT-PCR kit provides a quality system that offers the versatility of a one-step or two-step protocol.

Features and Benefits

- Greater transcription lengths than other reverse transcriptases, generating cDNA up to 14.1 kb
- Higher sensitivity for detecting low abundance messages. eAMV is able to transcribe RNA undetectable to other reverse transcriptases
- Transcribes through difficult secondary structure better than other reverse transcriptases
- Increased sensitivity, specificity and yield from JumpStart AccuTaq LA DNA Polymerase for long and accurate amplification

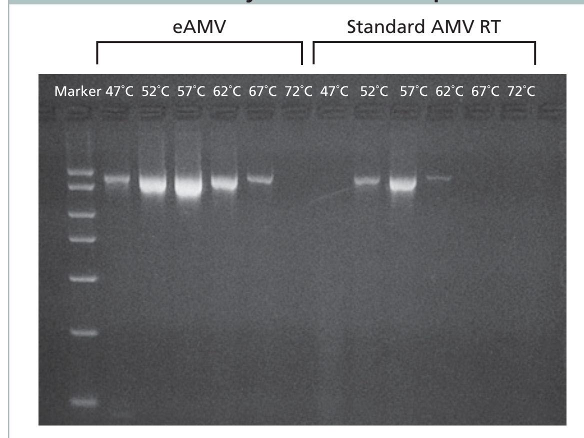
Only eAMV Detects Low Abundance Message



Only eAMV detects low abundance message. RT-PCR was performed on human phospholipase A_2, a low abundance RNA, (Lanes 2-7) and human HPRT, a medium abundance RNA (Lanes 8-13), using eAMV, RNase H-reduced AMV, and RNase H-minus M-MLV. Duplicate RT reactions were performed for each enzyme using 50 µg of HeLa poly(A)+ RNA and reactions from each enzyme were pooled together before PCR. Two µl of cDNA was used for each PCR reaction.

Lane 1: DNA marker
Lanes 2 and 3: RT-PCR using eAMV RT
Lanes 4 and 5: RT-PCR using RNase H-reduced AMV
Lanes 6 and 7: RT-PCR using RNase H-minus M-MLV
Lanes 8-13: Same enzyme order as Lanes 2-7

More Robust Activity at Elevated Temperatures



More robust activity at elevated temperatures. RT-PCR was performed on 1.7 kb TMV transcript containing difficult secondary structure. The primer used for the RT reaction is located in a region with extensive secondary stucture, making reverse transcription at an elevated temperature necessary.

Components: eAMV Reverse Transcriptase
JumpStart AccuTaq LA DNA Polymerase
Random Nonamers and Anchored Oligo(dT)$_{23}$ Primers
10× Reaction Buffers
10 mM dNTP Mix
Ribonuclease Inhibitor
PCR Grade Water

Storage: –20 °C
Shipped in dry ice

Ordering Information

Cat. No.	Product Description	Quantity
HSRT100	eAMV HS RT-PCR Kit Sufficient for 100 reactions	1 kit

DNase I, Amplification Grade

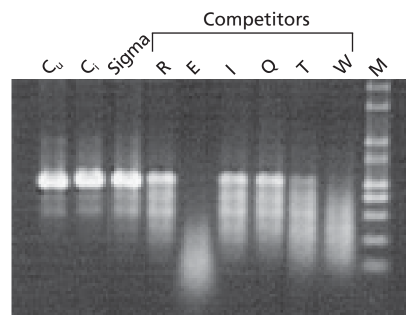
Because PCR can amplify even a single molecule of DNA, RNA samples should be treated with DNase I before RT-PCR, and control reactions should be run without reverse transcriptase to check for amplification of contaminating DNA. DNase I digests double and single-stranded DNA into oligo- and mononucleotides. DNA is removed from RNA preparations in a 15 minute digestion at room temperature using the Reaction Buffer provided. The DNase I is then inactivated by heat inactivation in the presence of Stop Solution. Heating also denatures hairpins in the RNA, so the RNA can be used directly in reverse transcription.

Many commercial DNase I formulations are contaminated with residual RNases. This RNase contamination can destroy or degrade valuable RNA samples prior to reverse transcription. Laboratory comparisons have shown that Sigma's Amplification Grade DNase I demonstrates lower levels of RNase activity than that of several leading molecular biology product suppliers.

Features and Benefits

- Ideal for eliminating DNA from RNA preparations prior to sensitive applications such as RT-PCR
- Lowest RNase activity available
- Includes optimized 10× Reaction Buffer and Stop Solution

Sigma Amplification Grade DNase I Has the Lowest RNase Activity



Sigma amplification grade DNase I has the lowest RNase activity. For Sigma DNase I, and for each competitor's DNase I, the following assay was completed: 1 µg of a 1.9 kb *in vitro* transcription product was incubated with 1 unit of the respective DNase I at 37 °C for 1 hour and analyzed on a 1% agarose gel.
C_u = unincubated control (RNA in buffer without DNase, kept on ice).
C_i = incubated control (RNA in buffer without DNase, incubated at 37 °C for 1 hour).

Note: To determine the effectiveness of DNase I treatment, control PCR reactions should be run without reverse transcriptase to check for amplification of contaminating DNA.

Components: DNase I, 1,000 units
10× Reaction buffer, 1 ml
Stop Solution, 1 ml

Unit definition: One unit completely digests 1 µg of plasmid DNA to oligonucleotides in 10 min at 37 °C

Storage: –20 °C
Shipped in wet ice

Ordering Information

Cat. No.	Product Description	Quantity
AMPD1	Deoxyribonuclease I, Amplification Grade	1 kit

AMV Reverse Transcriptase

from Avian Myeloblastosis Virus

AMV Reverse Transcriptase synthesizes DNA complementary to RNA templates (cDNA). A DNA primer complementary to the RNA template and a divalent cation, either Mg or Mn, are required for initiation of transcription. This enzyme is commonly used to make cDNAs from mRNA for eventual cloning or for use as probes.

Unit definition: One unit incorporates one nmol of TTP into TCA-precipitable material in 10 min using polyadenylic acid as template and oligo$(dT)_{12-18}$ as a primer

Concentration: 10,000-100,000 units per ml

Storage: –70 °C
Shipped in dry ice

References

1. Breathnach, R., et al., *Nature,* **270,** 314 (1977).
2. Tilghman, S.M., et al., *Proc. Natl. Acad. Sci. USA,* **75,** 1309 (1978).
3. Verma, I.M., *Biochim. Biophys. Acta,* **473,** 1 (1977).
4. Sambrook, J. and Russell, D.W., *Molecular Cloning: A Laboratory Manual, 3rd Ed.,* p. 8.48 (2001).
5. Okayama, H. and Berg, P., *Molec. Cell. Biol.,* **2,** 161 (1982).
6. Berger, S.L., et al., *Biochemistry,* **22,** 2365 (1985).
7. Leis, J.P., et al., *Proc. Natl. Acad. Sci. USA,* **70,** 466 (1973).
8. Champoux, J.J., et al., *J. Virol.,* **49,** 686 (1984).

Ordering Information

Cat. No.	Product Description	Quantity
R9376	AMV Reverse Transcriptase	500 units 1,000 units

M-MLV Reverse Transcriptase

Recombinant, expressed in Escherichia coli

M-MLV (Moloney Murine Leukemia Virus) Reverse Transcriptase is a DNA polymerase that uses single-stranded RNA, DNA or an RNA-DNA hybrid (using a primer) to synthesize a complementary DNA strand. M-MLV is used for the preparation of cDNA libraries or for first strand cDNA synthesis for use in RT-PCR reactions.

The enzyme is purified from *E. coli* expressing the *pol* gene of M-MLV on a plasmid.

Components: M-MLV Reverse Transcriptase
10× M-MLV Reverse Transcriptase Buffer with DTT

Unit definition: One unit incorporates 1 nmol of TTP into acid-precipitable material in 10 min at 37 °C

Concentration: 200 units per µl

Storage: –20 °C
Shipped in wet ice

References

1. Howland, P., et al., Positive- and negative-acting promoter sequences regulate cell type-specific expression of the rat synapsin I gene. *Mol. Brain Res.* **11,** 345-353 (1990).
2. Gerard, G.F. and D'Alessio, J.M., Buwell, M., Enzymes of Molecular Biology *Meth. Mol. Biol.* Totowa, N.J. **16,** 73 (1993).
3. Gerard, G.F., et al., Influence on stability in *Escherichia coli* of the carboxy-terminal structure of cloned Moloney murine leukemia virus reverse transcriptase *DNA* **5,** 271-279 (1986).

Ordering Information

Cat. No.	Product Description	Quantity
M1302	M-MLV Reverse Transcriptase	40,000 units

Oligo$(dT)_{23}$, Anchored

The Anchored Oligo $(dT)_{23}$ Primers have 23 thymidine residues and one G, C or A residue (the anchor) at the 3' end. This anchor ensures that the oligo$(dT)_{23}$ primer binds at the beginning of the message such that there are no long regions of unusable sequence. Thus, anchored oligo$(dT)_{23}$ primers may provide an advantage over standard oligo(dT) primers when generating cDNA from poly$(A)^+$ RNA.

0.1 ml is sufficient for 100 RT-PCR reactions (as described in the Technical Bulletin for Catalog Numbers HSRT100 and HSRT20).

Concentration: 70 µM

Storage: –20 °C
Shipped in wet ice

Ordering Information

Cat. No.	Product Description	Quantity
O4387	Oligo$(dT)_{23}$, Anchored	0.1 ml