

## RT-PCR Product Listing

Catalog Number	Product Description	Page
<a href="#">A4464</a>	eAMV Reverse Transcriptase	17
<a href="#">STR1</a>	eAMV First Strand Synthesis Kit	18
<a href="#">HSRT20</a>	eAMV HS RT-PCR Kit	19
<a href="#">HSRT100</a>	eAMV HS RT-PCR Kit	19
<a href="#">J3520</a>	JumpStart RED HT RT-PCR Kit	20
<a href="#">AMPD1</a>	Deoxyribonuclease I, Amplification Grade	21
<a href="#">R9376</a>	AMV Reverse Transcriptase	22
<a href="#">M1302</a>	M-MLV Reverse Transcriptase	22
<a href="#">O4387</a>	Oligo(dT) <sub>23</sub> , Anchored	22
<a href="#">R7647</a>	Random Nonamers	22

## 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

**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)<sub>12-18</sub> 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).

Cat. No.	Product Description	Quantity
<a href="#">A4464</a>	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 (RNA Inspector Kit, Catalog Number INSP1) chosen at different distances from the poly (A)<sup>+</sup> 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

## RT-PCR

### 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)<sup>+</sup> 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)<sub>12-18</sub> 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)<sub>23</sub>, 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).

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

# RT-PCR

## eAMV™ HS RT-PCR Kits

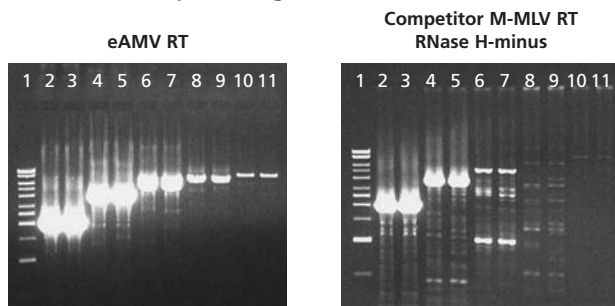
*The best choice for amplification of difficult templates and low abundance messages*

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

### Greater Transcription Lengths than M-MLV RT RNase H-minus

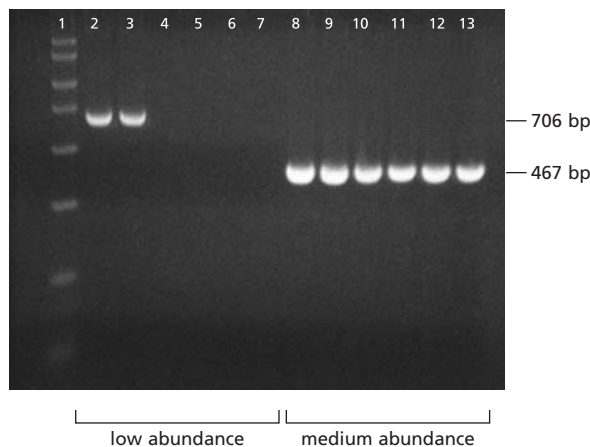


eAMV offers superior performance in length and yield over M-MLV RNase H-minus enzymes when transcribing long cDNA.

Different poly (A)<sup>+</sup> RNA were used as a template in a two-step RT-PCR.

- Lane 1: 1 kb Ladder  
 Lanes 2, 3: 2 kb Pol  
 Lanes 4, 5: 3.5 kb Pol  
 Lanes 6, 7: 5.3 kb TSC-2  
 Lanes 8, 9: 6.8 kb Pol  
 Lanes 10, 11: 8.9 kb APC

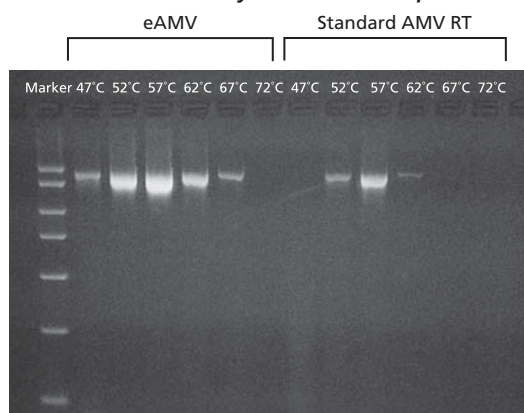
### Only eAMV Detects Low Abundance Message



RT-PCR was performed on human phospholipase A2, 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)<sup>+</sup> 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



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 structure, making reverse transcription at an elevated temperature necessary.

**Components:** eAMV Reverse Transcriptase  
 JumpStart AccuTaq LA DNA Polymerase  
 Random Nonamers and Anchored Oligo(dT)<sub>23</sub> Primers  
 10× Reaction Buffers  
 10 mM dNTP Mix  
 Ribonuclease Inhibitor  
 PCR Grade Water

**Storage:** -20 °C  
 Shipped in dry ice

Cat. No.	Product Description	Quantity
HSRT20	eAMV HS RT-PCR Kit Sufficient for 20 reactions	1 kit
HSRT100	eAMV HS RT-PCR Kit Sufficient for 100 reactions	1 kit

## RT-PCR

### JumpStart™ RED HT RT-PCR Kit

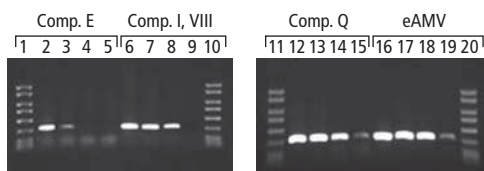
A novel high throughput solution for gel-based gene expression profiling. The JumpStart RED HT RT-PCR Kit is designed for high throughput gel-based RT-PCR applications and is optimized for end-point detection of target RNA in a complex sample matrix. The kit contains a combination of Sigma's Enhanced Avian Myeloblastosis Virus Reverse Transcriptase (eAMV-RT™) and JumpStart REDTaq™ DNA Polymerase.

#### Features and Benefits

- Greater transcription efficiency
- Enhanced sensitivity and specificity
- Direct sample loading after PCR
- Maximum flexibility and minimum optimization

#### eAMV Has Superior Performance and Thermal Stability

The sensitivity to low abundance message provided by eAMV is critical for comparative gene expression studies. eAMV has superior processivity and thermal stability compared to other reverse transcriptases. These unique features make it the ideal enzyme for detection of low abundance RNA, especially those with difficult secondary structure.



RT-PCR was performed on total RNA-HELA cells serially diluted from 1000 ng to 1 ng. Reactions were performed with  $\beta$  actin primers according to suppliers' protocols.

Lanes 1, 10, 11, 20: PCR Marker

Lanes 2, 6, 12, 16: 1000 ng

Lanes 3, 7, 13, 17: 100 ng

Lanes 4, 8, 14, 18: 10 ng

Lanes 5, 9, 15, 19: 1 ng

**Components:** eAMV Reverse Transcriptase  
JumpStart REDTaq DNA Polymerase  
eAMV RT 10× Buffer  
10× PCR Buffer without MgCl<sub>2</sub>  
Magnesium Chloride  
10 mm Deoxynucleotide Mix  
PCR Grade Water  
Oligo dT Primers  
Ribonuclease Inhibitor  
Random Nonomers

**Storage:** -20 °C  
Shipped in wet ice

Cat. No.	Product Description	Quantity
J3520	JumpStart RED HT RT-PCR Kit	40 reactions 200 reactions

## DNase I, Amplification Grade

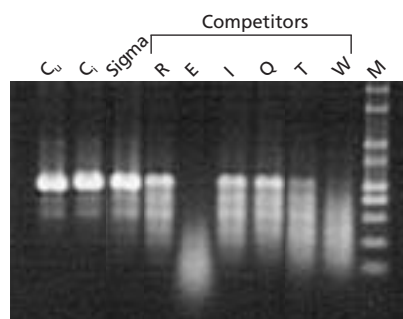
Because PCR can amplify even a single molecule of DNA, RNA samples should be treated with DNase I before RT-PCR, and control reactions should be run without reverse transcriptase to check for amplification of contaminating DNA. DNase I digests double and single-stranded DNA into oligo- and mononucleotides. 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



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

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

## RT-PCR

### 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)<sub>12-18</sub> as a primer

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

**Storage:** -70 °C  
Shipped in dry ice

#### References

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

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

- 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).
- Gerard, G.F. and D'Alessio, J.M., Buwell, M., Enzymes of Molecular Biology *Meth. Mol. Biol.* Totowa, N.J. **16**, 73 (1993).
- 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).

Cat. No.	Product Description	Quantity
M1302	M-MLV Reverse Transcriptase	40,000 units 200,000 units

### Oligo(dT)<sub>23</sub>, Anchored

The Anchored Oligo (dT)<sub>23</sub> 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)<sub>23</sub> primer binds at the beginning of the message such that there are no long regions of unusable sequence. Thus, anchored oligo(dT)<sub>23</sub> primers may provide an advantage over standard oligo(dT) primers when generating cDNA from poly(A)<sup>+</sup> 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

Cat. No.	Product Description	Quantity
O4387	Oligo(dT) <sub>23</sub> , Anchored	0.1 ml

### Random Nonamers

Random Nonamers are random sequences of nine deoxyribonucleotides (9-mers) which may be used as universal primers in first strand cDNA synthesis, cDNA library construction, RT-PCR and other applications.

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

**Concentration:** 50 µM

**Storage:** -20 °C  
Shipped in wet ice

Cat. No.	Product Description	Quantity
R7647	Random Nonamers	0.1 ml