

ParaScript Reverse Transcriptase

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-008 | Version 1.0 | April 2026

Cat. No.	EV-MOL-008	Size	1,000 U / 5,000 U	Storage	-20°C
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1. Overview

ParaScript Reverse Transcriptase (EV-MOL-008) is an engineered Moloney Murine Leukemia Virus Reverse Transcriptase produced in *E. coli* Rosetta(DE3). Four thermostabilizing mutations (R116K, D200N, T330P, L603W) extend the operational temperature range to 55°C, enabling reverse transcription of RNA templates with extensive secondary structure. The D524A mutation reduces RNase H activity by ~90%, improving full-length cDNA yield from long mRNA templates. The 674 amino acid, ~75 kDa enzyme is the standard reagent for two-step and one-step RT-PCR, cDNA library construction, and RNA-seq library preparation.

EV-MOL-008 is compatible with oligo(dT), random hexamer, and gene-specific primers. Each lot is tested for reverse transcriptase activity, RNase H activity level, and nuclease contamination before release.

2. Catalytic Mechanism

ParaScript RT catalyzes RNA-dependent DNA synthesis using a primer:template hybrid. The enzyme extends from a 3'-OH primer terminus, incorporating dNTPs complementary to the RNA template. The reduced RNase H activity (D524A mutation) allows synthesis of long cDNA products before template degradation, while residual RNase H activity aids template clearance for second-strand synthesis. The thermostabilizing mutations (R116K, D200N, T330P, L603W) maintain enzyme structure and activity at 42–55°C, where most RNA secondary structures are denatured.

3. Substrate Specificity

Substrate Type	Efficiency	Conditions
Polyadenylated mRNA (with oligo dT)	Very High	42–50°C, 30–60 min
Total RNA (with random hexamers)	High	25°C 5 min, then 42–50°C 30–60 min
Target mRNA (gene-specific primer)	Very High	50–55°C, 30 min; highest specificity
GC-rich or structured mRNA	High	55°C, 60 min; may add 5% DMSO

4. Reaction Conditions & Protocol

4.1 Recommended Reaction Setup

Component	Volume
RNA template (up to 5 µg total RNA or 500 ng mRNA)	x µL
Primer (oligo dT, random hexamers, or gene-specific; 10 µM)	1 µL
5x ParaScript RT Buffer (EV-MOL-008-RB)	4 µL
dNTPs (10 mM each)	1 µL
RNase Inhibitor (optional; 40 U/µL)	0.5 µL
ParaScript RT (EV-MOL-008)	1 µL (50 units)
Nuclease-free water	to 20 µL

- Primer annealing: 65°C 5 min, snap-cool on ice; then add remaining components
- Reverse transcription: 42°C for 15 min, then 50°C for 30 min (two-step temperature ramp recommended)
- Enzyme inactivation: 85°C for 5 min
- For one-step RT-PCR: 50°C 30 min RT, 95°C 2 min inactivation, then PCR cycling with EV5™ or Pfu

5. Unit Definition

One unit (U) is defined as the amount of ParaScript Reverse Transcriptase required to incorporate 1 nmol of dTMP into acid-insoluble material in 10 minutes at 37°C in a 50 µL reaction containing poly(A)-oligo(dT) template-primer and 200 µM [methyl-³H]-dTTP.

6. Quality Control

Test	Specification
Purity (SDS-PAGE)	>95% (single band at ~75 kDa)
Molecular Weight	~75 kDa (SDS-PAGE)
Concentration	50 U/µL
RT Activity	Full-length cDNA of 3 kb mRNA in 30 min at 50°C
RNase H Activity	Reduced (≤10% of wild-type MMLV-RT at 37°C)
Thermostability	Active at 55°C for 60 min; >80% activity retained
Exonuclease Contamination	No detectable degradation (λDNA, 200 U, 4 h, 37°C)
Endonuclease Activity	No nicking of supercoiled pUC19 (200 U, 4 h, 37°C)
RNase Activity (contamination)	No degradation of 5 µg RNA (200 U, 2 h, 37°C)

pH (formulation buffer)	8.1–8.5
Sterility	No microbial growth (7-day incubation)

7. Storage & Stability

- **Storage temperature:** -20°C (avoid repeated freeze-thaw; aliquot upon receipt)
- **Supplied in:** 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 50% glycerol
- **Stability:** 24 months from date of manufacture when stored correctly
- **Shipping:** On dry ice

8. Applications

- Two-step RT-PCR: first-strand cDNA synthesis followed by PCR amplification
- One-step RT-PCR in a single-tube format
- Full-length cDNA synthesis from mRNA with high GC content or secondary structure
- cDNA library construction from total RNA or poly(A)⁺ RNA
- Gene expression quantification by RT-qPCR
- RACE (Rapid Amplification of cDNA Ends)
- Transcriptome sequencing (RNA-seq) library preparation

9. Troubleshooting

Problem	Possible Cause	Suggested Action
Truncated cDNA products	RNA secondary structure or RNase contamination	Use 50–55°C; verify RNA integrity by gel before RT; add RNase inhibitor
Low cDNA yield	Degraded RNA or suboptimal primer	Use RIN ≥7 RNA; confirm primer T _m is appropriate for selected temp
High background in RT-qPCR	Genomic DNA contamination	DNase I treat RNA before RT; run no-RT controls
No product in one-step RT-PCR	Enzyme inhibition by carry-over reagents	Ensure PCR buffer is compatible; add RNase inhibitor to reaction

10. Safety Information

This product is intended for research use only. Handle in accordance with standard laboratory safety guidelines. Refer to the accompanying Safety Data Sheet (SDS) for full hazard information. Avoid ingestion, inhalation, or contact with eyes and skin. Dispose of in accordance with local, state, and federal regulations.

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