Evo™ Reverse Transcriptase

(Cat# M1173-25, -100; 200 U/µl; 20 µl rxns; Store at -20°C)

I. Introduction:

Evo™ Reverse Transcriptase is an optimized and evolved (Evo™) mutational derivative of the original Reverse Transcriptase (RTase) enzyme representing the best-performing RTase on the market. This enzyme catalyzes the synthesis of complementary DNA strands from single-stranded RNA or DNA templates. Due to a series of mutations introduced within the RNase H domain of this enzyme, there is no detectable RNase H activity associated with the enzyme. The lack of RNase H activity helps to eliminate RNA degradation during first-strand cDNA synthesis, resulting in better yield and length of cDNA synthesized (up to 9 kb). Furthermore, Evo™ RTase contains an additional fidelity enhancing subunit which drastically enhances accuracy in reverse transcription. **BioVision's Evo™ Reverse Transcriptase is the ultimate solution to synthesize cDNA from only 0.1 pg of RNA**.

II. Application:

- Synthesizing cDNA from ssRNA
- DNA primer extension
- Sequencing dsDNA
- Constructing cDNA library
- Producing template for use in RT PCR
- Labelling 3'-end of duplex DNA via end filling reactions
- Generating probes for hybridization

III. Package Contents:

Components	M1173-25 (25 x 20 µl rxns)	M1173-100 (100 x 20 µl rxns)	Part Number
Evo™ Reverse Transcriptase (200 U/µl)	25 µl	100 µl	M1173-XX-1
5X RT buffer	150 µl	600 µl	M1173-XX-2

IV. User Supplied Reagents and Equipments:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Oligo(dT) (10 µM) or Random Primers (10 µM)
- dNTPs (10 mM)
- RNaseOFF Ribonuclease Inhibitor (40 U/µl)
- Total RNA or poly(A) + mRNA

V. Shipment and Storage:

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly. Avoid repeated freeze-thaw cycles to retain maximum performance. Briefly centrifuge small vials prior to opening.

VI. Unit Definition:

- One unit is defined as the amount of enzyme required to incorporate 1 nmol of deoxynucleotide into acid-precipitable material in 10 min at 37ºC using poly(A) and Oligo(dT) as template and primer respectively.

VII. Primer Selection:

- Oligo(dT) are oligonucleotides that anneal to the 3'-poly(A) + mRNA. Therefore, only mRNA or total RNA templates with 3'-Poly(A) tails are used in cDNA synthesis.
- Random Primers are oligonucleotides that anneal at non-specific sites of RNA templates. Therefore, all forms of RNA can be used in cDNA synthesis.
- Gene-Specific Primers are oligonucleotides that are designed to anneal to the specific site of a target gene.

VIII. Protocol (First Strand cDNA Synthesis):

Reverse transcription reactions should be assembled in a RNase-free environment. The use of "clean", automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thaw RNA templates and all reagents on ice. Mix each solution by vortexing gently.
2. Prepare the following reaction mixture on ice.

Components	Volume	Final Concentration
Total RNA or poly(A) + mRNA	Variable	1 ng - 2 µg/rxn 1 pg - 2 ng/rxn
Oligo(dT) (10 µM) or Random Primers (10 µM) or Gene-Specific Primer	1 µl 1 µl Variable	0.5 µM 0.5 µM 10 - 15 nM
dNTP Mix (10 mM each)	1 µl	500 µM
Water, Nuclease-free	Up to 14.5 µl	

Optional: Heat mixture to 65°C for 5 min and incubate on ice for at least 1 min. Collect all components by a brief centrifugation

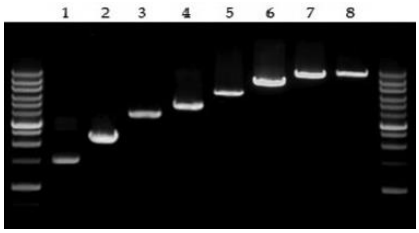
3. Add the following**:**

Components	Volume	Final Concentration
5X RT Buffer	4 µl	1X
RNaseOFF Ribonuclease Inhibitor (40 U/µl)	0.5 µl	20 U/rxn
Evo™ RTase (200 U/µl)	1 µl	200 U/rxn

4. Mix components well and collect all components (20 µl) by a brief centrifugation. Incubate the tube at 25°C for 10 min if using Random Primers. Omit this incubation if Oligo(dT) or Gene-Specific Primer is used.
5. Perform cDNA synthesis by incubating the tube for either 15 min (for QPCR) or 50 min (for PCR) at 42°C.

6. Stop reaction by heating it at 85°C for 5 min. Chill on ice. The newly synthesized first strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

IX. Sensitivity:



Elongation ability of Evo™ Reverse Transcriptase: L1:0.5 kb; L2:1.0 kb; L3:1.5 kb; L4:2.6 kb; L5:3.3 kb; L6:4.8 kb; L7:6.5 kb; L8:8.7 kb. PCR amplification using human cDNA (35 cycles) followed by electrophoresis on 1% agarose gels.

X. General Notes:

1. Both poly(A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
2. RNA samples must be free of genomic DNA contamination.
3. Unlike Oligo(dT) priming, which requires little optimization, the ratio of Random Primers to RNA is often critical in terms of the average length of cDNA synthesized. A higher ratio of Random Primers to RNA will result in a higher yield of shorter (~500 bp) cDNA, whereas a lower ratio will lead to longer cDNA products.
4. To remove RNA complementary to the cDNA, add 1 µl (2 U) of E. coli RNase H and incubate at 37°C for 20 min.

XI. Related Products:

BV Product Name	BV Cat. No.
Two Step RT PCR Kits	M1160-M1161
One Step RT PCR Kits	M1162-M1163
First Strand cDNA Synthesis Kits	M1164-M1167
First Strand cDNA Synthesis Supermixes	M1167-M1169
All-In-One RT Mastermixes	M1170-M1172
Reverse Transcriptases	M1173-M1174
One Step Jade™ QRT PCR Kits	M1175-M1182
One Step Taqman QRT PCR Kits	M1183-M1190

FOR RESEARCH USE ONLY! Not to be used on humans.