Novo™ Reverse Transcriptase

(Cat# M1174-100; 100 rxns; Store at -20 °C)

I. Introduction:

Novo™ Reverse Transcriptase is a Moloney-Murine Leukemia Virus Reverse Transcriptase with genetic modifications to abolish RNase H activity to achieve thermal stability. This special mutant enzyme offers higher cDNA yields, longer cDNA up to 12 kb, and is able to perform under high temperatures (50-55 °C), facilitating the elimination of secondary structures associated with GC-rich RNA templates. Novo™ Reverse Transcriptase is formulated with BioVision's RNaseOFF Ribonuclease Inhibitor offering improved resistance to oxidation compared to the high oxidation-sensitive human RNase inhibitors. RNaseOFF is stable even under very low concentrations of DTT (< 1 mM), making it the best choice for ultimate RNA protection.

II. Application:

- Synthesizing cDNA from ssRNA
- DNA primer extension
- Sequencing dsDNA
- Constructing cDNA library
- Constructing libraries for serial analysis for gene expression (SAGE)
- Synthesizing cDNA in rapid amplification of cDNA ends (3′ & 5′ RACE)
- 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	M1174-100 (100 rxns)	Part Number
Novo™ Reverse Transcriptase	100 µl	M1174-XX-1
5X RT buffer	400 µl	M1174-XX-2

IV. User Supplied Reagents and Equipments:

- PCR Tubes
- Pipettes
- Nuclease-free Water
- Primers (forward and reverse)
- Oligo(dT) (10 µM) or Random Primers (10 µM)
- dNTPs (10 mM)
- Total RNA or poly(A) + mRNA

V. Shipment and Storage:

Store all components at -20 °C.

VI. Primer Selection:

- Oligo(dT) (10 µM) 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 (10 µM) 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 (2 µM) are oligonucleotides that are designed to anneal to the specific site of a target gene.

VII. Protocol:

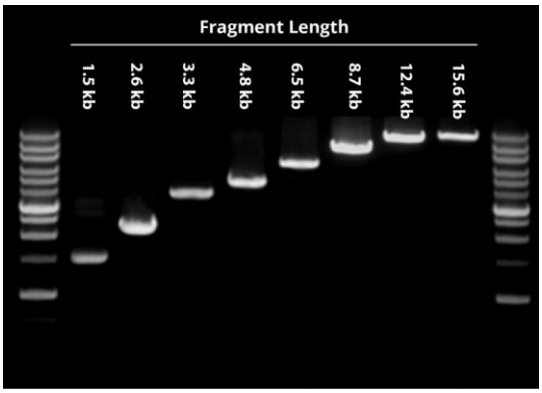
Reverse transcription reactions should be assembled in an RNase-free environment. The use of "clean", automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thoroughly thaw and mix individual components before use and assemble the reaction on ice.

Components	Volume
5X RT Buffer	4 µl
dNTP	1 µl
Primers	1 µl
Total RNA or poly(A) + mRNA	Variable (1 ng - 2 µg/rxn)
Novo™ RTase (200 U/µl)	1 ul
Nuclease-free Water	Up to 20 µl

2. Gently mix the reaction and briefly centrifuge.
3. Perform cDNA synthesis by incubating for either 15 min at 50-55 °C.
4. Optional: Stop the reaction by heating 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.

VIII. Sensitivity:



Novo™ Reverse Transcriptase can elongate RNA templates up to 15 kb in length. Novo™ Reverse Transcriptase was used in a reaction with a range of human RNA fragments. The resulting synthesized cDNA was followed by PCR and visualized on a 1% agarose gel.

IX. 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. For longer transcripts >9 kb, yields can be increased by incubating at 50-55 °C for 30-50 min.
3. RNA samples must be free of genomic DNA contamination.
4. 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. Due to the lower annealing temperature of Random Primers, incubate at 25 °C for 10 min to allow for primer annealing prior to reverse transcription.
5. To remove RNA complementary to the cDNA, add 1 µl of E. coli RNase H and incubate at 37 °C for 20 min.

X. Related Products:

BioVision Product Name	Cat. No.	Sizes
HiFidelity™ 2X PCR MasterMix	M1507	800 Rxns
ExpressTaq™ DNA Polymerase	M1504	400 Rxns
HiFidelity™ DNA Polymerase	M1505	400 Rxns
FireTaq™ DNA Polymerase	M1506	400 Rxns
ExpressTaq™ 2X PCR MasterMix	M1508	800 Rxns
HiFidelity™ One Step RT Kit	M1503	100 Rxns
ExpressTaq™ qPCR MasterMix	M1509	500 Rxns
Jade™ qPCR MasterMix	M1510	500 Rxns
Evo™ cDNA synthesis MasterMix	M1511	100 Rxns

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