Novo™ cDNA Kit

(Cat# M1165-25, -100; First Strand cDNA Synthesis Kit; Store at -20°C)

I. Introduction:

Novo™ cDNA Kit contains 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 °C-55 °C), facilitating the elimination of secondary structures associated with GC-rich RNA templates. Novo™ cDNA Kit (100 rxns) 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. Applications:

- 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. Key Features:

- Maximal flexibility in priming-oligo(dT), random primers or gene-specific primers
- Robust cDNA synthesis from any RNA template
- High reproducibility and excellent yield

IV. Package Contents (Novo™ cDNA Kit):

Components	M1165-25 (25 X 20 µl rxns)	M1165-100 (100 X 20 µl rxns)	Part Number
Novo™ RTase	25 µl	100 µl	M1165-XX-1
Oligo(dT) (10 µM)	40 µl	100 µl	M1165-XX-2
Random Primers (10 µM)	40 µl	100 µl	M1165-XX-3
dNTPs (10 mM)	40 µl	100 µl	M1165-XX-4
5X RT Buffer	150 µl	400 µl	M1165-XX-5
Nuclease-free H_2O	1 ml	2 X 1 ml	M1165-XX-6
RNaseOFF Ribonuclease Inhibitor (40 U/µl)	15 µl	-	M1165-XX-7

V. User Supplied Reagents and Equipment:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Total RNA or poly(A) + mRNA

VI. Shipment and Storage:

Store all components at -20°C in a non-frost-free freezer. Avoid repeated freeze-thaw cycles to retain maximum performance. Briefly centrifuge small vials prior to opening.

VII. Protocol:

RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR, and subsequent reaction analysis should be performed in separate areas. The use of "clean", automatic pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

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

Components	Volume
Total RNA or poly(A) + mRNA	Variable
Primers	1 µl
dNTP Mix	1 µl
Water, Nuclease-free	Up to 20 µl
5X RT Buffer	4 µl
RNaseOFF Ribonuclease Inhibitor (40 U/µl)*	0.5 µl (only for M1165-25, 25 rxns)
Novo™ RTase (200 U/µl)	1 µl

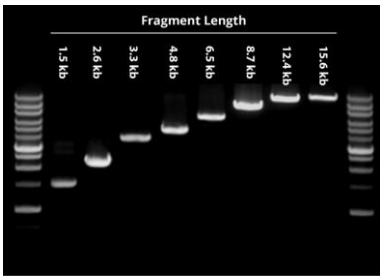
* RNaseOFF Ribonuclease Inhibitor is already provided in the formulation of Novo™ RTase (200 U/µl) for M1165-100, 100 rxns

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

VIII. General Notes:

- 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.
- For longer transcripts > 9 kb, yields can be increased by incubating at 50-55°C for 30-50 min.
- RNA samples must be free of genomic DNA contamination.
- 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.
- 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.

IX. 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. 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 Super Mixes	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

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