Novo™ Two Step RT Kit

(Cat# M1161-25, -100; Two Step RT PCR Kit; Store at -20°C)

I. Introduction:

Novo™ Two Step RT Kit is a highly efficient two step RT PCR kit that consists of two main components: **Novo™ cDNA Supermix (Cat# M1169-25, -100)** and **Robust Ready™ 2X PCR Master Mix with dye (Cat# M1131-200, -1000)**.

Novo™ cDNA Synthesis Supermix (Cat# M1169-25, -100) provides highly efficient and specific conversion of RNA to cDNA, even from low-abundance transcripts. Furthermore, the unique blend of random primers and oligo(dT) primers in this Super Mix ensures optimal representation of all transcript sequences in the cDNA product. Novo™ cDNA Synthesis Supermix is a proprietary mixture of all the materials required for first-strand cDNA synthesis in a 2X concentration (2X Reaction Mix) and the Novo™ Reverse Transcriptase. This optimized reaction mix contains RNaseOFF Ribonuclease Inhibitor, dNTPs, and a balanced concentration for Oligo(dT) and Random Primers. RNaseOFF Ribonuclease Inhibitor effectively protects RNA template from degradation. Oligo(dT) anneals selectively to the poly(A) tail of mRNAs. Random Primers do not require the presence of poly(A) and they are utilized for the transcription of mRNA 5'-end regions. The first-strand cDNA can be directly used as a template in PCR.

Novo™ Reverse Transcriptase (RTase) is a novel recombinant reverse transcriptase that exhibits much higher efficiency in the first-strand cDNA synthesis from RNA templates with secondary structures and high GC content. The Novo™ RTase is engineered to perform under high temperatures (50°C - 55°C), facilitating the elimination of secondary structures associated with GC-rich RNA templates. Due to this unique feature, Novo™ can synthesize full-length cDNA libraries from RNA templates up to 15 kb in length. In addition, Novo™ RTase has outstanding proofreading ability due to the presence of a fidelity-enhancing subunit, thus making this RTase an excellent choice for whole genome sequencing. **Overall, BioVision's cDNA Synthesis Supermix allows you to perform the first strand cDNA synthesis with minimal effort and maximum ease.**

Robust Ready™ 2X PCR Master Mix provides all the ingredients necessary for PCR in a premixed and optimized format that simplifies the PCR workflow. To set up a PCR reaction: add DNA template, primers and water. PCR products, amplified up to 6 kb in length with Robust Ready™ DNA Polymerase, contain a mixture of blunt ends and single base (A) 3' overhang. The error rate of this PCR amplification is $7.5x10^{-6}$ per nucleotide per cycle. The products can be used for direct T/A cloning, but its efficiency is not as high as PCR products amplified with Taq polymerase alone. Additionally, BioVision's Robust Ready™ Master Mix (with dye) includes an inert blue dye and a stabilizer too which allows the direct loading of the PCR product(s) onto an agarose gel.

BioVision's Novo™ Two Step RT PCR Kit is the most convenient and time-saving reaction set up for RT PCR of complex RNA templates including secondary structures and high GC content.

II. Application:

- Routine RT PCR amplification of RNA templates
- Reliable RT PCR based detection of gene expression

III. Key Features:

- Streamlined protocol suitable for high-throughput applications
- Ease of use with a simple set-up
- Excellent cDNA yields and robust PCR performance
- Suitable for complex RNA templates (secondary structures and high GC content)

IV. Package Contents (Novo™ cDNA Synthesis Supermix):

Components	M1161-25 (25 rxns)	M1161-100 (100 rxns)	Part Number
Novo™ RTase (200 U/µl)	25 µl	100 µl	M1161-XX-1
2X Reaction Mix	300 µl	1.2 ml	M1161-XX-2
Robust Ready™ 2X PCR Master Mix	4 ml (4 x 1 ml)	4 ml (4 x 1 ml)	M1161-XX-3

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. 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.

VII. Unit Definition (Novo™ cDNA Supermix)**:**

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

VIII. Storage Buffer (Novo™ cDNA Supermix)**:**

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01 % (v/v) NP-40, 50 % (v/v) glycerol

IX. A. Protocol (First Strand cDNA Synthesis using Novo™ cDNA Synthesis Supermix):

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.
3. Set up the following reaction mixture (10 µl or 20 µl reaction volume):

Components	Volume	Final Concentration
Total RNA or poly(A) + mRNA	Variable	1 ng - 2 µg/rxn 1 pg - 2 ng/rxn
2X Reaction Mix	10 µl	1X
Water, Nuclease-free	Up to 19 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.

4. Add the following**:**

Components	Volume	Final Concentration
Novo™ RTase (200 U/µl)	1 µl	200 U/rxn

5. 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.
6. Perform cDNA synthesis by incubating the tube for either 15 min (for QPCR) or 50 min (for PCR) at 42°C.
7. 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.

X. B. Protocol (Robust Ready™ 2X PCR Master Mix):

All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross-contamination. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended. Always keep the control DNA and other templates to be amplified isolated from the other components.

1. A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.
2. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

Components	Volume	Final Concentration
Robust Ready™ 2X PCR Master Mix	25 µl	1X
10 µM Forward Primer	1-2.5 µl	200-500 nM
10 µM Reverse Primer	1-2.5 µl	200-500 nM
Template DNA (from Step A)	~100 ng	~2 ng/ µl
Water, Nuclease-free	up to 50 µl	-

We recommend preparing a master mix for multiple reactions to minimize reagent loss and enable accurate pipetting.

3. Mix contents of tube and centrifuge briefly.
4. Incubate tube in a thermal cycler at 94°C for 3 min to completely denature the template.
5. Perform 30-35 cycles of PCR amplification as follows:
Denature: 94°C for 30 sec; Anneal: 45-72°C for 30 sec; Extend: 72°C for 1 min/1 kb template
6. Incubate for an additional 5 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
7. Analyze the amplified products by agarose gel electrophoresis and visualize by ethidium bromide or other DNA staining. Use appropriate molecular weight standards.

XI. 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.

XII. 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

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