Evo™ cDNA Kit (gDNA Removal)

(Cat# M1166-100; First Strand cDNA Synthesis Kit; Store at -20°C)

I. Introduction:

Evo™ cDNA Kit (with gDNA Removal) is a convenient and efficient system for first strand cDNA synthesis, now with a genomic DNA removal step included. The presence of contaminating genomic DNA (gDNA) in RNA preparations is often the cause of signification problems for downstream applications, leading to false-positive signals and misinterpretation of gene expression levels. Effective elimination of gDNA is therefore the most reliable method to ensure accurate experimental results.

The Genomic DNA (gDNA) Removal Kit provided will effectively remove contaminating gDNA from the sample in under 10 min, eliminating the need for heating or organic extraction which can cause damage to the RNA template. Following this step, the gDNA free RNA can be reverse-transcribed directly, using the Evo™ cDNA Synthesis Kit. The comprehensive set of RT reagents included with this kit offers maximum flexibility with respect to priming methods and reaction optimization for first-strand cDNA synthesis. With the added capability of gDNA removal, this improved system now generates the highest quality cDNA suitable for a wide range of downstream applications.
Note: Upon completion of the first-strand cDNA synthesis, the cDNA product can be directly applied as a template in a standard PCR/QPCR.

BioVision's Evo™ cDNA Kit offers a convenient and complete system for the elimination of contaminating genomic DNA in RNA preparations through to the synthesis of high quality cDNA for downstream applications.

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. Key Features:

- Quick ds and ssDNA digestion in under 10 minutes
- Maximal RNA protection-No heating or organic extraction required
- Flexibility in priming-oligo(dT), random primers or gene-specific primers
- Robust cDNA synthesis from any RNA template
- High reproducibility and excellent cDNA yield

IV. Package Contents (Evo™ cDNA Kit (gDNA Removal)):

Components	M1166-100 (100 X 20 µl rxns)	Part Number
Reaction Mix (4X)	200 µl	M1166-XX-1
Reaction Stopper (5X)	200 µl	M1166-XX-2
Evo™ RTase (200 U/µl)	100 µl	M1166-XX-3
Oligo(dT) (10 µM)	160 µl	M1166-XX-4
Random Primers (10 µM)	160 µl	M1166-XX-5
dNTPs (10 mM)	160 µl	M1166-XX-6
RNaseOFF Ribonuclease Inhibitor (40 U/µl)	60 µl	M1166-XX-7
5X RT Buffer	60 µl	M1166-XX-8
Nuclease-free H_2O	2 X 1 ml	M1166-XX-9

V. User Supplied Reagents and Equipment:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- Primers
- 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. Protocol:

All 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 template RNA on ice. Thaw all reagents at room temperature and spin briefly to collect residual liquid from the sides of the tubes. Keep the thawed reagents on ice.
2. Prepare the gDNA removal and subsequent reverse transcription reaction on ice:

Components	Volume
RNA Template	Up to 2 µg
Reaction Mix (4X)	2 µl
Water, Nuclease-free	Up to a total volume of 8 µl

Incubate at 42°C for 2 min or room temperature for 5 min, then add:

Reaction Stopper (5X)	2 µl

The purified RNA is ready for first-strand cDNA synthesis. Set-up the reverse transcription reaction by adding the components below into the Tube. *Optional step: Heat the reaction to 65°C for 5 min followed by on ice for 1 min.*

Components	Volume
5X RT Buffer	4 µl
Oligo(dT) (10 µl) or Random Primers (10 µM) or Gene-Specific Primer	1 µl 1 µl Variable*
dNTP Mix (10 mM)	1 µl
RNaseOFF Ribonuclease Inhibitor (40 U/µl)	0.5 µl
Evo™ RTase (200 U/µl)	1 µl
Nuclease-free H_2O	Up to a total volume of 20 µl

Incubate at 25°C for 10 min if using Random Primers (Omit this incubation if Oligo(dT) or Gene-Specific Primer is used), then incubate for either 15 min (for QPCR) or 50 min (for PCR) at 42°C. Inactivate the reaction at 85°C for 5 min. Chill on ice. **** Final concentration should be 10-15 nM.***

3. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or long term storage at -20°C.

VII. 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.
- 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.
- To remove the RNA complementary to cDNA, add 1 µl (2 U) of E. coli RNase H and incubate at 37°C for 20 min.

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

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