Evo™ RT Mastermix (gDNA Removal)

(Cat# M1171-100; Genomic DNA removal kit and 5X All-In-One RT Mastermix; Store at -20°C)

I. Introduction:

Evo™ RT Mastermix (with genomic DNA Removal Kit) is a 5X all-in-one RT Mastermix that provides a convenient and highly efficient method for first-strand cDNA synthesis with an additional genomic DNA removal step included. The presence of contaminating genomic DNA (gDNA) in RNA preparations is often a significant problem 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 Removal Kit provided will effectively remove contaminating gDNA from the sample in under 10 minutes, without any heating or organic extraction steps which can result in damage to the RNA template. The treated gDNA-free RNA can then be directly reverse-transcribed into cDNA using the 5X All-In-One RT Mastermix.

The 5X RT Mastermix is a ready-to-use formulation of all the reagents necessary for first-strand cDNA synthesis. Coupled together, this complete system provides the ultimate convenience in generating high-quality cDNA suitable for a wide range of downstream applications. N*ote: 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™ RT Mastermix (with gDNA removal) offers removal of contaminating genomic DNA from your samples and reverse transcription of RNA in 30 min.

II. Application:

- Generation of templates for use in RT PCR and QRT PCR
- cDNA synthesis from ssRNA
- cDNA library construction
- Generation of probes for hybridization
- DNA primer extension

III. Key Features:

- Efficient ds and ssDNA digestion in under 10 min
- Maximal RNA protection- No heating or organic extraction steps required
- Fewer handling errors, with only 1 liquid transfer step for reverse transcription
- Streamlined protocol suitable for high-throughput application
- Simple set-up for any RNA template
- High reproducibility and excellent cDNA yield

IV. Package Contents (Evo™ RT Mastermix (gDNA Removal)):

Components	M1171-100 (100 X 20 µl rxns)	Part Number
Reaction Mix (4X)	200 µl	M1171-XX-1
Reaction Stopper (5X)	200 µl	M1171-XX-2
5X All-In-One RT Mastermix	400 µl	M1171-XX-3
Nuclease-free H_2O	1 ml	M1171-XX-4

V. User Supplied Reagents and Equipment:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- RNA Template

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:

Both gDNA removal and reverse transcription reactions should be assembled in a RNase-free environment. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended. Keep the RNA on ice to minimize RNA degradation.

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 following reactions for gDNA removal and subsequent reverse transcription 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 the following to the tube:

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:

Components	Volume
5X All-In-One RT Mastermix	4 µl
Water, Nuclease-free	6 µl
Total Reaction Volume	20 µl

Incubate at 25°C for 10 min, then incubate at 42°C for another 15 min (if downstream application is QPCR) or 50 min (for PCR). Inactivate the reaction at 85°C for 5 min. Chill on ice.

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

VII. General Notes:

- 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 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.