Evo™ RT Mastermix

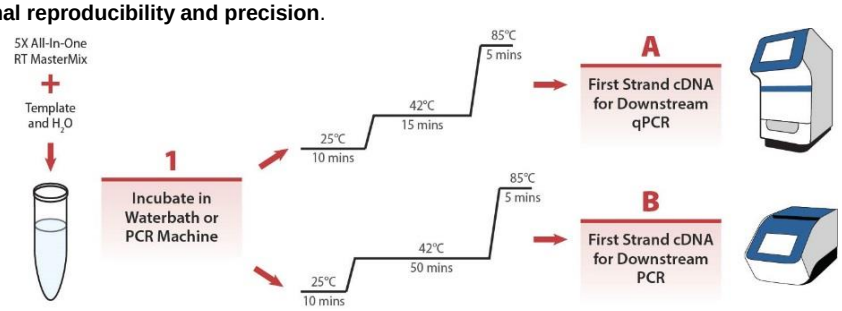
(Cat# M1170-25, -100, -200; 5X All-In-One RT Mastermix; Store at -20°C)

I. Introduction:

Evo™ RT Mastermix is a 5X All-In-One RT Mastermix and is a convenient, ready-to-use formulation of all the reagents necessary for first-strand cDNA synthesis with the exception of the template. This optimized, 5X concentrated reaction mastermix contains BioVision's proprietary **Evo™ Reverse Transcriptase** (Evo™ RTase), RNaseOFF Ribonuclease Inhibitor, dNTPs and a finely balanced ratio of Oligo(dT)s and Random Primers. Programmed to catalyze the synthesis of complementary DNA strands from single-stranded RNA/DNA templates, Evo™ RTase is an enhanced, engineered version of the native RTase enzyme from Moloney Murine Leukemia Virus.

An array of strategic mutations including those for the abrogation of RNase H activity, endow Evo™ RTase with its superior catalytic prowess. Nullifying the RNase H activity which is intrinsic to the native RTase helps prevent RNA degradation during first-strand cDNA synthesis resulting in higher yields and an increase in the achievable length of synthesized cDNA. Evo™ RTase also contains a fidelity-enhancing subunit which ensures superior accuracy in reverse transcription. A vital component, the RNaseOFF Ribonuclease Inhibitor serves to effectively protect the RNA template from any possible degradation by RNases. With respect to options for primers, while the Oligo(dT)s selectively anneal to the Poly(A) tail of mRNAs, the Random Primers, with their non-specific nature of annealing allow for the use of any type of RNA as the template.
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™ RT Mastermix is the convenient solution from RNA template to cDNA in just 15 min in a completely hassle-free manner yet with exceptional reproducibility and precision.



II. Applications:

- 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:

- Reduction in handling errors with only 1 liquid transfer step
- Streamlined protocol suitable for high-throughput applications
- Simple set-up for any RNA template
- High reproducibility and excellent yield

IV. Package Contents (Evo™ RT Mastermix):

Components	M1170-25 (25 rxns)	M1170-100 (100 rxns)	M1170-200 (200 rxns)	Part Number
5X All-In-One RT Mastermix	50 µl	200 µl	400 µl	M1170-XX-1
Nuclease-free H_2O	1 ml	1 ml	2 x 1 ml	M1170-XX-2

V. User Supplied Reagents and Equipment:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- 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. Primer Information:

Oligo(dT)s are oligonucleotides that anneal to the 3'-Poly(A) tail of mRNAs. Therefore, the utility of Oligo(dT) is restricted to case scenarios where only mRNA or total RNA templates with 3'-Poly(A) tails are used for cDNA synthesis. On the other hand, since Random Primers anneal at non-specific sites within RNA template(s), they can be used generically for all forms of RNA as template for cDNA synthesis.

VIII. Protocol:

Reverse transcription reactions should always be conducted in an RNase-free environment. The use of clean, automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended.
1. Thaw RNA templates and the 5X All-In-One RT Mastermix on ice. Mix solutions gently but thoroughly.

2. Prepare the following reaction mixture in a PCR tube on ice:

Components	Reaction Volume		Final Concentration
	10 µl	20 µl	
Total RNA or poly(A) + mRNA	Variable	Variable	2 pg - 2 µg/20 µl rxn 0.01 pg - 2 ng/20 µl rxn
5X all in one RT Mastermix	2 µl	4 µl	1X
Nuclease-free H_2O	Up to 10 µl	Up to 20 µl	-

3. Mix the components well and collect by brief centrifugation. Incubate the tube at 25°C for 10 min.
4. Perform cDNA synthesis by incubating the tube for either 15 min (for QPCR) or 50 min (for PCR) at 42°C.
5. Stop the reaction by heating it at 85°C for 5 min followed by chilling on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

VII. 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. 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. 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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