BIO BASIC
Quality-Affordable Research

Product information

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5X All-In-One RT MasterMix

Catalog #: HRT100-20G
Size: 100 Rxns
Storage: -20°C

ATTENTION: New Protocol with Improved Performances and Removal of Genomic DNA!

Components and Variants:

Components	
5X All-In-One RT MasterMix	400 ul
Nuclease-Free H2O	2 x 1ml
Size	**100rxns**

Description:

5X All-In-One RT MasterMix 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 proprietary Reverse Transcriptase, 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, the Reverse Transcriptase 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 Reverse Transcriptase RTase with its superior catalytic prowess. Nullifying the RNase H activity which is intrinsic to native Reverse Transcriptase RTase helps prevent RNA degradation during first-strand cDNA synthesis resulting in higher yields and an increase in the achievable length of synthesized cDNA. Reverse Transcriptase RTase also contains a fidelity-enhancing subunit which ensures superior accuracy in reverse transcription. A vital component, the 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.

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.

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 ul	20 ul	
Total RNA or Poly(A) + mRNA	Variable	Variable	2.00 pg - 2.00 ug/20 ul rxn
			0.01 pg - 2.00 ng/20 ul rxn
5X All-In-One RT MasterMix	2 ul	4 ul	1X
Nuclease-free H2O	to 10 ul	to 20 ul	-

3. Mix the components well and collect by brief centrifugation.
4. Perform cDNAsynthesis by incubating the tube at 37°C for 15 min, followed by 60°C for 10 min.
5. Stop the reaction by heating it at 85°C for 5 mins 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.

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 ul (2 U) of E. coli RNase H and incubate at 37°C for 20 mins.

Storage and Stability:

Store all components at -20°C in a non-defrost cycle freezer.
All components are stable for 1 year from the date of shipment when stored and handled properly.