Apex™
Chemicals and Reagents
Exclusively from Genesee Scientific

Apex 5X cDNA Synthesis Supermix

Catalog #: 42-702B / 42-702C / 42-702D
Size: 50rxns / 100rxns / 500rxns
Storage: -20˚C

Components and Variants

Components	42-702B	Catalog #: 42-702C	42-702D
5X cDNA Supermix	200µl	400µl	2,000µl
Nuclease-free H_2O	1ml	2 x 1ml	10 x 1ml
# of 20µl Reactions	50	100	500

General Description

5X cDNA Supermix 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 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 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 a 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 cDNA Supermix on ice. Mix solutions gently but thoroughly.
2. Prepare the following reaction mixture in a PCR tube on ice:

Components	Reaction Volume		Final Concentration
	10µl	20µl	
Total RNA or Poly(A) + mRNA	Variable	Variable	2.00pg - 2.00ug/20µl rxn
			0.01pg - 2.00ng/20µl rxn
5X All-In-One RT MasterMix	2µl	4µl	1X
Nuclease-free H_2O	to 10µl	to 20µl	-

3. Mix the components well and collect by brief centrifugation.
4. Perform cDNA synthesis by incubating the tube at 37˚C for 15 minutes, followed by 60˚C for 10 minutes.
5. Stop the reaction by heating it at 85˚C for 5 minutes 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 1µl (2U) of E. coli RNase H and incubate at 37˚C for 20 minutes.

Storage

Store all components at -20˚C in a non-defrost cycle freezer.

Related Products

0.2ml 8-Strip PCR Tubes, Natural

With individual attached flat caps
Cat #: 27-125U

Apex qPCR GREEN Master Mix

All-in-one master mixes for dye (SYBR)-based detection - just add primers and DNA!
Cat #: 42-116PG

Apex 2X Taq Master Mix, Red

Just add template, primers and water
Cat #: 42-138