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EasyScript Plus™ cDNA Synthesis Supermix

Store at -20°C

Part No.	Components	Cat. No.	
		G453	G454
RT-2	EasyScript Plus™ RTase (200 U/µl)	25 µl	100 µl
RT-8	2X Reaction Mix	300 µl	1.2 ml
RT-0	Nuclease-free H_2O	1 ml	2 x 1 ml
Size		**25 rxns**	**100 rxns**

Product Description

EasyScript Plus™ Reverse Transcriptase is a novel recombinant reverse transcriptase that exhibits much higher efficiency in the first-strand cDNA synthesis from RNA templates with secondary structures and high GC content. The EasyScript Plus™ Reverse Transcriptase is engineered to perform under high temperatures (50°C - 55°C), facilitating the elimination of secondary structures associated with GC-rich RNA templates. Due to this unique feature, EasyScript Plus™ can synthesize full-length cDNA libraries from RNA templates up to 15 kb in length. In addition, EasyScript Plus™ Reverse Transcriptase has outstanding proofreading ability due to the presence of a fidelity-enhancing subunit, thus making this RTase an excellent choice for whole genome sequencing.

abm's cDNA Synthesis Supermix is a proprietary mixture of all materials required for first-strand cDNA synthesis in a 2X concentration. This optimized reaction mix contains RNaseOFF Ribonuclease Inhibitor, dNTPs, and a balanced concentration for Oligo(dT) and Random Primers. RNaseOFF Ribonuclease Inhibitor effectively protects RNA template from degradation. Oligo(dT) anneals selectively to the poly(A) tail of mRNAs. Random Primers do not require the presence of poly(A) and they are utilized for the transcription of mRNA 5'-end regions. The first-strand cDNA can be directly used as a template in PCR.

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C using poly(A) and Oligo(dT) as template and primer, respectively.

Storage Buffer

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01 % (v/v) NP-40, 50 % (v/v) glycerol.

Storage Conditions

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.

Protocol

Reverse transcription 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 RNA templates and all reagents on ice. Mix each solution by vortexing gently.
2. Prepare the following reaction mixture on ice:

Components	Volume	Final Concentration
Total RNA or poly(A) + mRNA	Variable	1 ng - 2 µg/rxn 1 pg - 2 ng/rxn
2X Reaction Mix	10 µl	1X
Nuclease-free H_2O	Up to 19 µl	-

3. **Optional:** Heat mixture to 65°C for 5 mins and incubate on ice for at least 1 min. Collect all components by a brief centrifugation.
4. Add the following:

Components	Volume	Final Concentration
EasyScript Plus™ RTase (200 U/µl)	1 µl	200 U/rxn

5. Mix components well and collect all components (20 µl) by a brief centrifugation. Incubate the tube at 25°C for 10 mins. Perform cDNA synthesis by incubating the tube for either 15 mins (for qPCR) or 50 mins (for PCR) at 50°C.
6. Stop the reaction by heating it at 85°C for 5 mins. Chill 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. 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.
4. To remove RNA complementary to the cDNA, add 1 µl (2 U) of *E. coli* RNase H and incubate at 37°C for 20 mins.