		EasyScript™			
		Reverse Transcriptase		cDNA Synthesis Kit	
Part No.	**Components**	**G231**	**G232**	**G233**	**G234**
RT-1	EasyScript™ RTase (200 U/µl)	25 µl	100 µl	25 µl	100 µl
RT-3	Oligo(dT) (10 µM)	-	-	40 µl	160 µl
RT-4	Random Primers (10 µM)	-	-	40 µl	160 µl
RT-5	dNTPs (10 mM)	-	-	40 µl	160 µl
RT-6	RNaseOFF Ribonuclease Inhibitor (40 U/µl)	-	-	15 µl	60 µl
RT-7	5X RT buffer	150 µl	600 µl	150 µl	600 µl
RT-0	Nuclease-free H_2O	-	-	1 ml	2 x 1 ml
	Size	25 rxns	100 rxns	25 rxns	100 rxns

Product Description

EasyScript™ Reverse Transcriptase is an optimized mutational derivative of the original RTase enzyme representing the best-performing RTase on the market. This enzyme catalyzes the synthesis of complementary DNA strands from single-stranded RNA or DNA templates. Due to a series of mutations introduced within the RNase H domain of this enzyme, there is no detectable RNase H activity associated with the enzyme. The lack of RNase H activity helps to eliminate RNA degradation during first-strand cDNA synthesis, resulting in better yield and length of cDNA synthesized. Furthermore, EasyScript™ RTase contains an additional fidelity-enhancing subunit which drastically enhances accuracy in reverse transcription.

EasyScript™ cDNA Synthesis Kit contains all materials required for first-strand cDNA synthesis, with the choice of using either Oligo(dT) and/or Random Primers. The Oligo(dT) anneals selectively to the Poly(A) tail of mRNAs. Random Primers do not require the presence of poly (A) and can be used for the transcription of mRNA 5'-end regions. Gene-specific primers may also be used with the kit. The recombinant RNaseOFF Ribonuclease Inhibitor, supplied with the kit, effectively protects RNA template from degradation. 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.

Primer Selection

Oligo(dT) are oligonucleotides that anneal to the 3'-Poly(A) + mRNA. Therefore, only mRNA or total RNA templates with 3'-Poly(A) tails are used in cDNA synthesis.

Random Primers are oligonucleotides that anneal at non-specific sites of RNA templates. Therefore, all forms of RNA can be used in cDNA synthesis.

Gene-Specific Primers are oligonucleotides that are designed to anneal to the specific site of a target gene.

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 Condition

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 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
Oligo(dT) (10 µM) or Random Primers (10 µM) or Gene-Specific Primer	1 µl 1 µl Variable	0.5 µM 0.5 µM 10 - 15 nM
dNTP Mix (10 mM each)	1 µl	500 µM
Nuclease-free H_2O	Up to 14.5 µl	-

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

Components	Volume	Final Concentration
5X RT Buffer	4 µl	1X
RNaseOFF Ribonuclease Inhibitor (40 U/µl)	0.5 µl	20 U/rxn
EasyScript™ RTase (200 U/µl)	1 µl	200 U/rxn

4. Mix components well and collect all components (20 µl) by a brief centrifugation. Incubate the tube at 25°C for 10 mins if using Random Primers. Omit this incubation if Oligo(dT) or Gene-Specific Primer is used.

5. Perform cDNA synthesis by incubating the tube for 50 mins at 42°C.

6. Stop 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.*