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RTScript Reverse Transcriptase
RTScript cDNA Synthesis Kit

Store at -20°C

		RTScript			
		Reverse Transcriptase		cDNA Synthesis Kit	
Part No.	Components	PCR-231	PCR-232	PCR-233	PCR-234
RT-1	RTScript 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	RNaseRID Ribonuclease Inhibitor	-	-	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
RTScript 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, RTScriptRTase contains an additional fidelity-enhancing subunit which drastically enhances accuracy in reverse transcription.

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

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.

3. Add the following:

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

4. Mix components well and collect all components (20 µl) by a brief centrifugation.
5. 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.
6. Perform cDNA synthesis by incubating the tube for either 15 mins (for qPCR) or 50 mins (for PCR) at 42°C.
7. 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.

For laboratory research only. Not for clinical applications.
For technical questions, please email us at technical@biocrede.com or visit our website at www.biocrde.com