RScriptHot cDNA Synthesis Kit

Cat. No. G594EU

Store at -20°C.

Product Description

RScriptHot cDNA Synthesis Kit is the first kit in **the world** that allows for efficient and robust cDNA synthesis at 60°C. All other cDNA synthesis kits on the market are optimized for the reaction to be performed at 42-55°C, which is sufficient for most RNA template, but fails on more difficult templates. **abm** is the only company in the world to have a reverse transcriptase engineered to offer superior cDNA synthesis performance with even the most challenging RNA samples due to its **incredible thermostability at 60-72°C**.

The enzyme is a mutational derivative of Moloney-Murine Leukemia Virus Reverse Transcriptase, that can reverse transcribe low abundance or degraded RNA, and has significantly better resistance to contaminating inhibitors such as reagents used during RNA extraction and contaminants from biological samples. High processivity and sensitivity allow for rapid cDNA synthesis of full-length cDNA fragments in a fraction of the time of leading competitors. RScriptHot is formulated with **abm**'s RNaseOFF Ribonuclease Inhibitor offering improved resistance to oxidation compared to the high oxidation-sensitive human RNase inhibitors. RNaseOFF is stable even under very low concentrations of DTT (< 1 mM), making it the best choice for ultimate RNA protection.

This kit provides a comprehensive set of reagents necessary to generate high quality cDNA and offers the most flexibility in respect to priming methods and reaction optimization. Both random primers and oligo(dT) are included for a choice of general priming strategies and as alternatives to gene-specific primers.

Product Component	Quantity	Part No.
RScript Hot RTase	100 rxn (100 µl)	P104EU
5X RT Buffer	400 µl	P110
Oligo(dT) (10 µM)	100 µl	P106
Random Primers (10 µM)	100 µl	P107
dNTP (10 mM)	100 µl	P108
Nuclease-Free H_2O	2 x 1.0 ml	P100

Protocol

RT reactions should be assembled in an RNase-free environment. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thoroughly thaw and mix individual components before use, and assemble reaction on ice.

Component	Volume
5X RT Buffer	4 µl
dNTP	1 µl
Primers	1 µl
Total RNA or poly(A) + mRNA	Variable (1 ng - 2 µg/rxn)
RScript Hot RTase	1 µl
Nuclease-free H_2O	up to 20 µl

2. Gently mix the reaction and briefly centrifuge.
3. Perform cDNA synthesis by incubating for 15 minutes at 60°C.
4. Optional: Stop the reaction by heating at 85°C for 5 minutes. 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

- 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.
- For longer transcripts >9 kb, yields can be increased by incubating at 60°C for 30-50 minutes.
- RNA samples must be free of genomic DNA contamination.
- 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. Due to the lower annealing temperature of Random Primers, incubate at 25°C for 10 minutes to allow for primer annealing prior to reverse transcription.

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com