OneScript® Plus cDNA Synthesis Kit

Cat. No. G236

Store at -20°C.

Product Description

OneScript® Plus cDNA Synthesis Kit contains a Moloney-Murine Leukemia Virus Reverse Transcriptase with genetic modifications to abolish RNase H activity to achieve thermal stability. This special mutant enzyme offers higher cDNA yields, longer cDNA up to 12 kb, and is able to perform under high temperatures (50°C - 55°C), facilitating the elimination of secondary structures associated with GC-rich RNA templates. OneScript® Plus 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.

Product Component	Quantity	Part No.
OneScript® Plus RTase	100 rxn (100 µl)	P105
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)
OneScript® Plus 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 50-55°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 50-55°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