OneScript® Plus Reverse Transcriptase

Cat. No. G237

Store at -20°C.

Product Description

OneScript® Plus Reverse Transcriptase is 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

Additional Materials Required (not supplied)

- Primers, see Primer Selection notes
- dNTP (10 mM)
- Nuclease-free H_2O

Primer Selection

- **Oligo(dT) (10 µM)** 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 (10 µM)** 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 (2 µM)** are oligonucleotides that are designed to anneal to the specific site of a target gene.

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.

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