Product information

Product Name: PoScript Reverse Transcriptase

Catalog No: VN60RT, VN61RT

Packing Size: 1 x 100 µl and 4 x 100 µl

Shipping Condition: Dry ice / Ice pack

Storage Condition: -20^{0}C

Product Description:

PoScript Reverse Transcriptase is a mutational derivative of Moloney-Murine Leukemia Virus Reverse Transcriptase. It is specifically designed to reverse transcribe low-abundance or degraded RNA, and it offers significantly better resistance to contaminants and inhibitors such as reagents used during RNA extraction and contaminants from biological samples.

This enzyme is highly processive and sensitive, which allows for rapid cDNA synthesis of full-length cDNA fragments in a fraction of the time of leading competitors. PoScript is the only engineered reverse transcriptase on the market that can offer superior cDNA synthesis performance with even the most challenging RNA samples due to its incredible thermostability at 60-75ºC. Therefore, it is the enzyme of choice for both daily or demanding RNA reverse transcription applications.

Additionally, PoScript reverse transcriptase is formulated to offer improved resistance to oxidation.

Product Features

- Thermostable from 60-75°C
- Transcribes degraded samples and resists inhibitors / contaminants
- Provides high cDNA yields from difficult or ultra-low RNA samples
- High yields of full-length cDNA up to 15 kb
- Efficient—complete cDNA synthesis in 15 to 30 minutes

Applications

- First strand cDNA synthesis for RT-PCR and real-time RT-PCR.
- Reverse transcription at elevated temperatures to reduce effects of secondary structure.
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays.
- Analysis of RNA by primer extension.

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.

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

5X RT Buffer		4 ul
dNTP Mix, 10 mM each		1 ul
Primers	Oligo(dT)(10 uM)	1 ul
	Random Primers (10 uM)	1 ul
	Gene-specific Primers (2 uM)	1 ul
Template RNA	Total or Poly(A) RNA	1 ng-2 ug
	Specific RNA	0.01 pg-500 ng
PoScript RTase		0.5-1 ul*
Water, nuclease-free		To 20 ul

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 -80°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.
- To remove RNA complementary to the cDNA, add 1 µl of *E. coli* RNase H and incubate at 37°C for 20 minutes.

Note: This product is for R&D use only

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