

GScript RTase

Cat. No.: MB303-0050

Size: 50 Reactions

Cat. No.: MB303-0010

Size: 10 Reactions

Store at -20°C

Description

The GScript RTase is recombinant M-MLV RTase expressed in *E. coli* and purified to homogeneity. It has lower RNase H activity and high thermal stability. The enzyme is widely used to synthesize first-strand cDNA at temperatures up to 55°C with increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. It can generate cDNA from 100 bp to 12 Kb.

Kit Content

Contents	MB303-0050	MB303-0010
GScript RTase (200 units/μl)	50 μl	10 μl
5X RT Buffer	250 μl	50 μl
0.1 M DTT	100 μl	20 μl

Quality Control

The quality of the GScript RTase is tested on a lot-to-lot basis to ensure consistent product quality.

Protocol

First-Strand cDNA Synthesis

- In a sterile microcentrifuge tube, first add:
 - RNA solution (10 pg~5 μg total RNA or 10 pg~500 ng mRNA)
 - 1 μl oligo(dT)₂₀ (50 μM), or other primers
 - 1 μl 10 mM dNTP Mix
 - nuclease-free H₂O to final volume of 13 μl
- Heat for 3-5 minutes at 65°C. Spin briefly and place promptly on ice.

3. Add:

- 4 μl 5X RT Buffer
- 1 μl 0.1 M DTT
- 1 μl RNase Inhibitor (10 U/μl)
- 1 μl GScript RTase (200 units/μl)
- final volume 20 μl

If generating cDNA longer than 5 kb at temperatures above 50°C using a gene-specific primer or oligo(dT)₂₀, the amount of GScript RTase may be raised to 400 U (2 μl) to increase yield.

- Incubate at 50°C for 30-60 minutes. Increase the reaction temperature to 55°C for gene-specific primer.
Reaction temperature may also be increased to 55°C for difficult templates or templates with high secondary.
- Inactivate enzyme at 70°C for 15 minutes.
- Store products at -20°C or proceed to PCR using 2 μl first-strand cDNA synthesis reaction mixture.
Amplification of some PCR targets (> 1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 μl (2 units) of *E. coli* RNase H and incubate at 37°C for 20 minutes.

Related Ordering Information

Cat. No.	Description	Package
MB101-0500	Taq DNA Polymerase	500 units
DN021-1000	10 mM dNTP Mix	1 ml
DM010-R500	1Kb DNA Ladder RTU	500 μl
LD001-1000	Novel Juice	1 ml

All products are for research use only.