

GScript RTase

Cat No. MB303-0010

Size: 10 Reactions

Store @ -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.

Component

| | |
|---------------|-------|
| GScript RTase | 10 µl |
| 5X RT Buffer | 50 µl |
| 0.1 M DTT | 20 µl |

First-Strand cDNA Synthesis

- In a sterile microfuge 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.
- 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.

Storage

Store all components at -20°C (non-frost-free). Thaw 5X RT Buffer, 0.1 M DTT at room temperature just prior to use and refreeze immediately.