GScript RTase

Cat No. MB303-0050
Size: 50 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	50 µl
5X RT Buffer	250 µl
0.1 M DTT	100 µl

First-Strand cDNA Synthesis

1. 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 H_2O to final volume of 13 µl
2. 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.
4. 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.
5. Inactivate enzyme at 70°C for 15 minutes.
6. 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 complememtary 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.