# GScript First-Strand Synthesis Kit

Cat. No.: SM305-0050 Size: 50 Reactions
Cat. No.: SM305-0010 Size: 10 Reactions
Store at -20°C

## Description

The GScript First-Strand Synthesis Kit is a recombinant M-MLV RTase expressed in *E. coli* and purified to homogeneity. It has a lower RNase H activity and a 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. The GScript RTase has a special activity with its secondary structure, rendering stabilization during the reverse transcription reaction.

## Kit Content

| Component | SM305-0050 | SM305-0010 |
|---|---|---|
| Oligo(dT)$_{20}$ (50 μM) | 50 μl | 10 μl |
| 5X 1st strand buffer | 250 μl | 50 ml |
| DTT (0.1 M) | 100 μl | 20 μl |
| dNTP mix (10 mM) | 50 μl | 10 μl |
| GScript RTase (200 U/ μl) | 50 μl | 10 μl |

## Quality Control

The quality of the GScript First-Strand Synthesis Kit is tested on a lot-to-lot basis to ensure consistent product quality.

## Required Materials

- Microcentrifuge tubes
- RiboINTM RNase inhibitor
- Water bath / Dry bath
- Nuclease-free $H_2O$

## 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) | X μl |
| Oligo(dT)$_{20}$ (50 μM) | 1 μl |
| dNTP mix (10 mM) | 1 μl |
| Nuclease-free $H_2O$ | to 13 μl |

2. Heat for 3-5 minutes at 65°C.
3. Spin briefly and place promptly on ice.
4. Add:

| | |
|---|---|
| 5X 1st strand buffer | 4 μl |
| DTT (0.1 M) | 1 μl |
| RiboINTM RNase Inhibitor (40U/μl) (optional)* | 0.25 μl |
| GScript RTase | 1 μl |
| make the final volume | to 20 μl |

*(Optional: If the RNA is less than 50ng, add RiboINTM RNase Inhibitor to remove RNase).

If generating a cDNA longer than 5 kb at temperatures above 50°C by 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.

5. Incubate at 50°C for 30-60 minutes. Increase the reaction temperature to 55°C for the gene-specific primer. Reaction temperature may also be increased to 55°C for difficult templates or templates with a high level of secondary structures.
6. Inactivate the enzyme at 70°C for 15 minutes.
7. Store products at -20°C or proceed to PCR using 2 μl of the 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

1. Store all components at -20°C (non-frost-free).
2. Thaw 5X 1st strand buffer and DTT (0.1 M) at the room temperature just prior to use and refreeze immediately.
3. Thaw the dNTP mix (10 mM) and Oligo(dT)20 on ice prior to use and refreeze immediately.

## Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when synthesizes a complementary DNA strand from RNA with the kit.

| Problem | Cause | Solution |
|---|---|---|
| No bands after analysis of amplified products | Procedural error in first-strand cDNA synthesis | Re-purify the RNA template if the nucleic acid appears degraded. |
| | RNase contamination | Use RNase Inhibitor in the first-strand reaction. |

| Problem | Cause | Solution |
|---|---|---|
| No bands after analysis of amplified products | Degraded template | Verify the integrity of the template by electrophoresis after incubation. Re-purify the RNA template if the nucleic acid appears degraded |
| Unexpected bands after electrophoretic analysis | Genomic DNA sequences related to the RNA template contaminate the RNA preparation | Pretreat RNA with DNase I before PCR Reaction. |
| | Nonspecific annealing of primers | Vary the annealing conditions. |

## Caution

1. During operation, always wear a lab coat, disposable gloves, and protective equipment.
2. All products are for research use only.