GScript One-Step RT-PCR Kit

Cat. No.: SM306-0050 Size: 50 Reactions
Cat. No.: SM306-0005 Size: 5 Reactions
Store at -20°C

Description

The GScript One-Step RT-PCR Kit contains all necessary reagents for both reverse transcription and PCR amplification to occur in a single reaction tube. Components for both cDNA synthesis and PCR are combined in a single tube, using gene-specific primers and target RNAs from either total RNA or mRNA. Reverse transcription automatically follows PCR cycling without additional steps. The GScript One-Step RT-PCR Kit consists of two major components: GScript Enzyme Mix and 5X Reaction Mix, provided a convenient format for highly sensitive and specific RT-PCR using any RNA template. Our proprietary RT-PCR buffer contains enhancer that optimizes the two reactions in a "single step". Together with a specially formulated RT-PCR buffer, this GScript One-Step RT-PCR kit offers the end-users an efficient, easy to use and reliable alternative to conventional. The GScript Enzyme Mix is for optimal cDNA synthesis and PCR amplification. The enzyme mix uses a mixture of M-MLV Reverse Transcriptase and Hotstart Taq DNA polymerase in an optimized reaction buffer.The 5X Reaction Mix consists of a proprietary buffer system optimized for reverse transcription and PCR amplification, Mg^{2+} optimized for universal use, deoxyribonucleotide triphosphates, and stabilizers. This convenient 5X format allows further addition of template and primer at any desired concentration. Sufficient reagents are provided for 50 amplification reactions of 50 µl each.

Kit Content

Component	SM306-0050	SM306-0005
GScript Enzyme Mix	100 µl	10 µl
5X Reaction Mix	550 µl	55 µl

Important Parameters

RNA

- High quality intact RNA is essential for successful full-length cDNA synthesis.
- RNA should be avoiding of any RNase contamination and aseptic conditions should be maintained.

Primers

- Gene specific primers (GSP) are recommended. Use of oligo(dT) or random primers are not recommended as they result in generation of non-specific products in the one-step procedure and the amount of RT-PCR product may be reduced.
- A final primer concentration of 0.2 µM for each primer is generally optimal. For best results, a primer titration using 0.15-0.5 µM is recommended. Primers should not be self-complementary or complementary to each other at the 3' ends.

Magnesium Concentration

- The magnesium is included in the 5X Reaction Mix at a final concentration of 2.3 mM, which works well for most RT-PCR

dNTPs Concentration

- dNTPs are included in the 5X Reaction Mix, which is optimal for most reactions.

Recommendations and Tips

- Keep all components, reaction mixes, and samples on ice. After preparation of the samples, transfer them to a pre-heated thermal cycler (45-55°C, depending on the cDNA step temperature) and immediately start the RT-PCR amplification program. Efficient cDNA synthesis can be accomplished in 15-30 minute incubation at 45-55°C.
- The annealing temperature should be 10°C below the melting temperature of the primers used.
- The extension time varies with the size of the amplicon (approximately 1 minute per 1 kb of amplicon).

Quality Control

The quality of the GeneDireX GScript One-Step RT-PCR Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Protocol

1. Program the thermal cycler so that cDNA synthesis is followed immediately with PCR amplification automatically.

Note: The following cycling conditions were established using a DNA Thermal Cycler and may have to be altered for other thermal cyclers. Efficient cDNA synthesis can be achieved in 15-30 minute incubation at 45-55°C. The optimal temperature for reverse transcription will depend on primer and target sequences. Remember that cycling conditions may have to be further optimized for different sequences. Annealing and extension steps are separate (three-step cycling).

2. Add the following to the microcentrifuge tubes placed on ice. Reaction cocktails can be made when multiple reactions are being assembled.

Components	Volume (µl)	Final
5X Reaction Mix	10	1X
Template RNA	x	1 pg - 1 µg
Sense Primer (10 µM)	1	0.2 µM
Anti-sense Primer (10 µM)	1	0.2 µM
GScript Enzyme Mix	2	–
Autoclaved distilled water	to 50	–

3. Gently mix and make sure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

Temperature	Time	
45-55°C	15-30 min	cDNA Synthesis
94°C	5 min	RTase Inactivation / Hotstart *Taq* Activation
94°C	15 sec	Denature
55-60°C	30 sec	Anneal
68-72°C	1 min/kb	Extension
72°C	5-10 min	Final Extension
4°C	∞	Hold

4. Analyze the amplification product.

Troubleshooting Guide

Problem	Possible cause	Possible solution
No amplification product	No cDNA synthesis	For the cDNA synthesis step, incubate at 45- 55°C.
	Not enough starting template RNA	Increase the concentration of template RNA; use 1 pg- 1 µg of total RNA.
	RNA has been damaged or degraded	Replace RNA if necessary.
Low specificity	Reaction conditions not optimal	Optimize magnesium concentration, primer, annealing temperature or extension time. Increase temperature of RT reaction to 55°C.
	Oligo(dT) or random primers used for first strand synthesis	Use gene-specific primers.
Unexpected bands after electrophoresis	RNA contamination with genomic DNA	Pre-treat RNA with DNase I.

Caution:

- During the operation, always wear the latex or vinyl gloves while handling reagents and RNA samples to prevent the RNase contamination.
- All products are for research use only.