GeneDireX®

GScript*ULTRA* First-Strand Synthesis Kit (Random primer)

Cat. No. MB310-H100 Size: 100 Reactions
Cat. No. MB310-H010 Size: 10 Reactions
Store at -20°C
Minimum shelf life: 1 year

Description

The GScript*ULTRA* First-Strand Synthesis Kit (Random primer) provides a convenient and sensitive of cDNA synthesis from RNA molecules by reverse transcription (RT). The kit consists of five major components: GScript*ULTRA* RTase, 5X 1st strand buffer, 0.1 M DTT, 50 µM Random Hexamers primer, and 10 mM dNTP (Deoxyribonucleotide triphosphates) mix. The GScript*ULTRA* RTase is a recombinant Moloney Murine Leukemia Virus (M-MLV) transcription polymerase 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 base pairs (bps) to 12 Kilo base pairs (kb). The 5X Reaction Mix buffer is optimized for reverse transcription. The DTT breaks the disulfide bonds, loosen the secondary structure of RNA, and helps in initiation for cDNA synthesis. Random Hexamers are short oligodeoxyribonucleotides of random sequences [d(N)6] that anneal to random complementary sites on a target RNA. The dNTP solution consists all four nucleotides (dATP, dCTP, dGTP, dTTP), suitable for use in cDNA synthesis.

Feature

- Time efficiency – included most reagents for reverse transcription reaction.

Application

- Downstream application for PCR to detection of expressed genes, examination of transcript variants, or generation of cDNA templates for cloning and sequencing.

Kit contents

Contents	MB310-H100	MB310-H010
Random Hexamers (50 µM)	100 µl	10 µl
5X 1st strand buffer	500 µl	50 µl
DTT (0.1 M)	100 µl	10 µl
dNTP mix (10 mM)	100 µl	10 µl
GScript*ULTRA* RTase	40 µl	4 µl

Quality Control

The quality of the GScript*ULTRA* First-Strand Synthesis Kit (Random primer) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Microcentrifuge tubes
- PCR instrument or water / Dry bath
- RNase-free H_2O
- RNase Inhibitor (10 U/µl)

GScript*ULTRA* First-Strand Synthesis Kit (Random primer) Protocol

1. Thaw the GScript*ULTRA* First-Strand Synthesis Kit (Random primer), template RNA, primers and RNase-free H_2O on ice. Mix each solution well.
2. Set up the following reaction mixture, and reaction cocktails can be made when multiple reactions are being assembled.

Components	Volume (µl)
Template RNA (10 ng~5 µg total RNA or 10 pg~500 ng mRNA)	Variable
Random Hexamers (50 µM)	1 µl
10 mM dNTP Mix	1 µl
Add RNase-free H_2O to	13.6 µl

3. Heat for 3-5 minutes at 65°C. Spin briefly and place promptly on ice.
4. Add the following component to the same tube:

Components	Volume (µl)
5X 1st strand buffer	4 µl
DTT (0.1 M)	1 µl
RNase Inhibitor (10 U/µl) (recommended)	1 µl
GScript*ULTRA* RTase	0.4 µl
Final volume to	20

Note: If generating cDNA longer than 5 kb at temperatures above 50°C using a gene-specific primer (GSP) or oligo(dT)$_{20}$, the amount of GScript*ULTRA* RTase may be raised to 2 µl to increase yield.

5. Incubate at 50°C for 30-60 minutes. Increase the reaction temperature to 55°C for GSP and difficult templates or templates with high secondary.
6. Inactivate enzyme at 70°C for 15 minutes.
7. Store products at -20°C or proceed to PCR. Using 2 µl first-strand cDNA synthesis reaction mixtures. 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 minute.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did cDNA synthesis with the kit.

Problem	Possible cause	Possible solution
Low yield of PCR products	Incomplete concentration of start materials	Use the appropriate method for the RNA preparation based on the amount of the starting materials.
	RNA degraded	Avoid repeated freeze / thaw cycles of the sample.
		Keep DNA preparations on ice or frozen in order to avoid the degradation.
	RNase contaminant	Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free enzymes, e.g.: RNase inhibitor.

Related ordering information

Cat. No.	Description	Size
MB101-0500	*Taq* DNA Polymerase	500 units
MB203-0100	OnePCR™	100 Reactions
DM010-R500	1 Kb DNA Ladder RTU	500 µl
NA017-0100	Total RNA Isolation Kit (Blood/ Cultured Cell/ Fungus)	100 Reactions
NA020-0100	Total RNA Isolation Kit (Plant)	100 Reactions
NA021-0100	Total RNA Isolation Kit (Tissue)	100 Reactions
RI001-2500	RiboIN RNase Inhibitor	2500 U

Caution:

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