Gene to Protein Pvt. Ltd.

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Compli-Script™ 1st strand cDNA synthesis Kit

Cat # ☐ **RNA005-50** ☐ **RNA005-100** ☐ **RNA005-50-S**
Pack Size: ☐ **50 Rxn ;** ☐ **100 Rxn;** ☐ **5 Rxn**
Storage: -20°C

Introduction

The Gene to Protein Compli-Script™ 1st strand cDNA synthesis kit is meant for both cDNA using Oligo dT or random primers from either total RNA or mRNA. The system uses MMLV Reverse Transcriptase with reduced RNase H activity in an optimized reaction buffer, and it can detect a wide range of RNA targets from 100 bp to > 6 kb. The amount of starting material can range from 20 ng to 1 µg of total RNA.

The enzyme can synthesize cDNA optimally at a temperature of 42°C, providing increased specificity, higher yields of cDNA, working efficiently on RNA having secondary structures, and generating more full-length product than other reverse transcriptases.

Kit content

S.No	Cat. No	Contents	5 RXN	50 RXN	100 RXN
1	RNA-1000	MMLV RT Enzyme (80 U/µL)	5 µL	50 µL	100 µL
2	RNA-900	5X Reaction Buffer mix	20 µL	200 µL	400 µL
3	RNA-600	Oligo dT_{21} (50µM)	2.5 µL	25 µL	50 µL
4	RNA-700	Random Nonamer (50µM)	2.5 µL	25 µL	50 µL
5	RNA-800	dNTP (10 mM)	5 µL	50 µL	100 µL
6	RNA-500	RNase inhibitor (40 U/µL)	1.25 µL	12.5 µL	25 µL
7	RNA-1200	Nuclease free water	1 mL	1 ml	2 ml
8	RNA-200	DTT (100mM)	5 µL	50µL	100µL

Application

- First strand cDNA synthesis
- Producing cDNA for PCR and Realtime PCR
- Gene expression validation of Microarray experiments
- RT-PCR validation of silencing by RNA interference

Guideline for RNA quality and quantity

- High-quality, intact RNA is essential for successful full length cDNA synthesis. For low copy-number genes or longer targets, use more starting material (>100 ng total RNA). Estimate RNA quality by Bioanalyser and Gel electrophoresis to evaluate quality
- RNA should be devoid of any RNase contamination and aseptic conditions should be maintained during isolation. Trizol (or any similar product) or any spin column-based RNA isolation kit may be used.

Procedure

Setting up Reaction mix: Total volume 20µL

S.No	Components	Volume
1	50 µM oligo$(dT)_{21}$ primer/50 µM Random Nanomer / 10 µM gene-specific reverse primer	0.5µL
2	Template RNA	100 ng– 1 µg mRNA
3	RNase-Free dH2O	Variable
	Total	12.75 µL

Spin briefly and heat for 5 min at 65°C and chill immediately on ice, followed by addition of undermentioned components:

S.No	Components	Volume
4	5X Reaction Buffer	4 µL
5	10 mM dNTP mix	1 µL
6	RNase Inhibitor	0.25 µL
7	MMLV Reverse Transcriptase (80U/µL)	1 µL
8	DTT (100 mM)	1 µL
	Total	20 µL

Spin briefly and incubate at 42°C* for 60 min
*The optimal temperature for reverse transcription depends on primer and target sequences. Also, incubation time can be varied from 30-60 min depending upon the size and complexity of gene.

Result

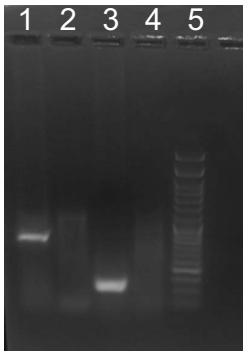

Figure: RT-PCR of a 180 bp and 400 bp gene fragment with 100ng of total RNA (Human Blood) using β-actin and GAPDH primers.
Lane 1 – 400 bp PCR product using GAPDH primers
Lane 2 – Negative control (NC).
Lane 3 – 180 bp PCR product using β-Actin primers
Lane 4 – NC
Lane 5 – 1kb ladder

Troubleshooting

Observation	Possible cause	Recommended action
No amplification product	No cDNA synthesis (temperature for Reverse transcription may be too high)	Lower temperature for Reverse transcription step
	RNase contamination	Maintainaseptic conditions; add RNase inhibitor. (Although Buffer already contains inhibitor)
	Not enough starting template RNA	Increase concentration of template RNA; use 100 ng t 1µg of total RNA
	RNA has been damaged or degraded	Replace RNA ifnecessary
	RT inhibitors are present in RNA	Remove inhibitors in RNA preparation by an additiona 70% ethanol wash. Note: Inhibitorsof RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate, and spermidine.
	Annealing temperature is high	Decrease temperature as necessary
	Extension time is short	Set extension time @ 60 sec/kb
	No. of cycles less	Increase cycle number
Low specificity	Reaction conditions not optimal	Optimize magnesium concentration
		Optimize primer
		Optimize theannealing temperature and extension tim
		Increase temperature of RT reaction
	Oligo(dT) or random primers used	Use only genespecific primers
Unexpected bands after electrophoretic analysis	Contamination from genomic DNA	Pretreat RNA with DNaseIbefore use
		Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary the mRNA to differentiate between amplified cDNA ar potential contaminating genomic DNA
	Nonspecific annealing of primers	Vary theannealing temperature
		Optimize the magnesium concentration for each template and primer combination
	Primer dimer	Redesign primers without complementary sequence a the 3'ends