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Compli-Amp™ One-Step RT-PCR Kit

Cat # ☐ RNA009-50 ☐ RNA009-100 ☐ RNA009-50-S
Pack Size: ☐ 50 Rxn ; ☐ 100 Rxn; ☐ 5 Rxn
Storage: -20°C

Introduction

The Gene to Protein One step RT PCR kit is meant for both cDNA synthesis and PCR amplification in a single tube using gene specific primers and targets genes from either total RNA or mRNA. The system uses a mixture of MMLV Reverse Transcriptase and Taq DNA Polymerase 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. There is no need to add reagents separately during the reaction and thus lowering the risk of contamination.
The Kit includes MMLV Reverse Transcriptase that has been engineered to reduce RNase H activity and provide increased thermal stability. The enzyme can synthesize cDNA at a temperature range of 42–50°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

Cat. No.	Contents	Pack size		Storage
		50 RXN	100RXN	
RNA-100	Enzyme mix	25µL	50 µL	-20℃
RNA-200	2X Reaction Buffer mix	500 µL	1000 µL	
RNA-300	Control RNA	30 µL	60 µL	
RNA-400	Control primer set	10 µL	20 µL	

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.

PCR Condition

- The thermal cycler should be preheated to 42-50°C, depending on the temperature selected for cDNA synthesis.
- Keep all components, reaction mixes, and samples on ice. After setting up reaction, transfer tube to the preheated thermal cycler and immediately start the RT-PCR program.
- Efficient cDNA synthesis can be accomplished in a 15–30-minute incubation at 42-50°C.

Procedure

Preparation for Reaction mix: Total volume 20µL

Components	Amounts
2X Reaction Buffer mix (RNA-200)	9.5 µL
Enzyme mix (RNA-100)	0.5 µL
Forward primer	0.5-10 µM
Reverse primer	0.5-10 µM
RNA template(10ng-1µg)	Variable
NFW	Variable
Total	20µL

Cycling Condition

cDNA synthesis and inactivation of Reverse transcriptase		Denature	Anneal	Extend	Final Extension (optional)
		35 PCR CYCLES			
42°C	95°C	94°C	55–65°C*	72°C	72°C
30min	10min	15sec	30sec	1min/kb	5min

1. *The optimal temperature for reverse transcription depends on primer and target sequences. Cycling conditions may have to be further optimized for different sequences.

2. **Control reaction:** Take 5 µL of Control template RNA and parallelly set up one reaction as above using 1.0 µL of Control primer set with rest of the components as identical.

Gel loding Protocol

1. Prepare your DNA sample for electrophoresis by mixing it with the appropriate volume of GreenTrack 6X Loading Dye. The recommended ratio is 1:5 (v/v), meaning for every 1 part of DNA sample, add 5 parts of loading dye.
2. Load your DNA sample onto an agarose gel.
3. Run electrophoresis according to your experiment's protocol.
4. Monitor the progress of the electrophoresis by observing the migration of the DNA through the gel.
5. Once the electrophoresis is complete, visualize the DNA by staining the gel with an appropriate dye.

Result

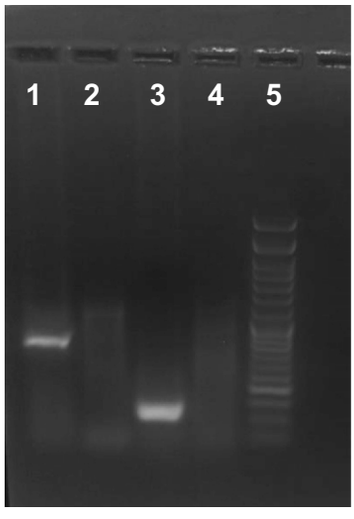

Figure One step RT-PCR of a 180 bp and 400 bp gene fragment with 100ng of total RNA (Human Blood) using β-actin and GAPDH primers.
Lane 1 – 400 bp PCR product using GAPDH primers
Lane 2 – Negative control (NC).
Lane 3 – 180 bp PCR product using β-Actin primers
Lane 4 – NC
Lane 5 – 1kb ladder

Troubleshoot

Observation	Possible cause	Recommended action
No amplification product	No cDNA synthesis temperature for Reverse transcriptio n may be too high)	Lower temperature for Reverse transcription step
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor. (Although Buffer already contains inhibitor)
	Not enough starting template RNA	Increase concentration of template RNA; use 100 ng to 1ìg of total RNA
	RNA has been damaged or degraded	Replace RNA if necessary
	RT inhibitors are present in RNA	Remove inhibitors in RNA preparation by an additional 70% ethanol wash. Note: Inhibitors of 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 the annealing temperature and extension time
		Increase temperature of RT reaction
	Oligo(dT) or random primers used	Use only gene-specific primers
Unexpected bands after electrophoretic analysis	Contamination from genomic DNA	Pretreat RNA with DNaseI before use
		Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating genomic DNA
	Nonspecific annealing of primers	Vary the annealing temperature
		Optimize the magnesium concentration for each template and primer combination
	Primer dimer	Redesign primers without complementary sequence at the 3'ends