Gene to Protein Pvt. Ltd.

Gene to Protein Pvt. Ltd.
www.genetoprotein.com
info@genetoprotein.com
800 GENOME, 800 GENETIC

Compli-Amp™ High fidelity Two step RT-PCR Kit

Cat # ☐ RNA007-50 ☐ RNA007-100 ☐ RNA007-50-S
Pack Size: ☐ 50 Rxn ; ☐ 100 Rxn; ☐ 5 Rxn
Storage: -20°C

Introduction

The Two-step High-Fidelity RT-PCR kit is a reverse transcription reagent kit designed to perform reverse transcription optimized for 2-step real-time RT-PCR (RT-qPCR). It contains a 5X reaction buffer, MMLV RT, Oligo dT, Random nanomers, RNase Inhibitor, Contro RNA and Primer (for control), and dNTP Mixture. The kit is suitable to synthesize template cDNA for real-time PCR efficiently in a short time. The cDNA obtained with this product can be used in both probe qPCR assay and intercalator qPCR assay.

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

S. No.	Cat. No.	Component	Amounts (50 Rxn)	Amounts (100Rxn)	Storage
1	RNA-500	RNase inhibitor (40 U/µL)	12.5 µL	25 µL	-20˚C
2	RNA-600	Oligo dT_{20} (50µM)	25 µL	50 µL	
3	RNA-700	Random Nonamer (50µM)	25 µL	50 µL	
4	RNA-800	dNTP (10 mM)	50 µL	100 µL	
5	RNA-900	RT-PCR Buffer (5X)	200 µL	400 µL	
6	RNA-1000	MMLV-RT Enzyme (80 U/µL)	12.5 µL	25 µL	
7	RNA-1100	Taq DNA Polymerase (5U/µL)	12.5 µL	25 µL	
8	RNA-1300	10X PCR Buffer	150 µL	300 µL	
9	GreenR ™ *(SyberGreen Equvalent)*		25 µL	50 µL	
10	RNA-1200	Nuclease free water	1 ml	2 ml	
11	RNA-300	Control RNA	30 µL	60 µL	
12	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 the 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.

RT Conditions

- 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 the reaction, transfer the tube to the preheated thermal cycler and immediately start the RT-PCR program.
- Efficient cDNA synthesis can be accomplished in a 30–60-minute incubation at 42-50°C.

Procedure

Step 1: (Reverse Transcription Step)

Setting up Reaction mix: Total volume 20µL

Components	Volume
50 µM oligo(dT_{20}) primer/50 µM Random Nonamer / 10 µM genespecific reverse primer	0.5µL
Template RNA	20 ng– 1 µg mRNA
RNase-Free dH2O	Variable
Total	**14.5 µL**

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

Components	Volume
5X Reaction Buffer	4 µL
10 mM dNTP mix	1 µL
RNase Inhibitor	0.25 µL
MMLV Reverse Transcriptase (80U/µL)	0.25 µL

Spin briefly and incubate at 42°C* for 60 min

*The optimal temperature for reverse transcription depends on primer and target sequences. Cycling conditions may have to be further optimized for different sequences. Also, the incubation time can be varied from 30-60 min depending upon the size and complexity of the gene

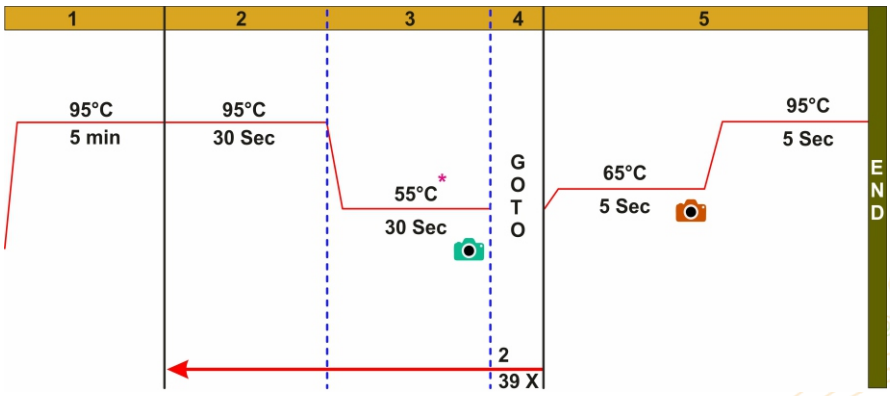
Control reaction: Take 5 µL of Control template RNA and parallelly set up one reaction as above and subject to PCR as below using 1.0 µL of Control primer set with the rest of the components as identical.

Step 2: (qPCR Step)

In a clean tube on ice, combine the following PCR reagents to a 25 µl final volume

Components	Volume
Taq DNA polymerase (5U/ µL)	0.25 µL
10X PCR Buffer	2.5 µL
dNTP(10mM)	2.5 µL
Forward Primer	5-10 µM
Reverse Primer	5-10 µM
GreenR™ (Sybergreen Equivalent)	0.5 µL
RT mix (from Step 1)	5-10 µL(variable)
RNase Free dH2O	Variable

PCR Cycling conditions for real-time PCR



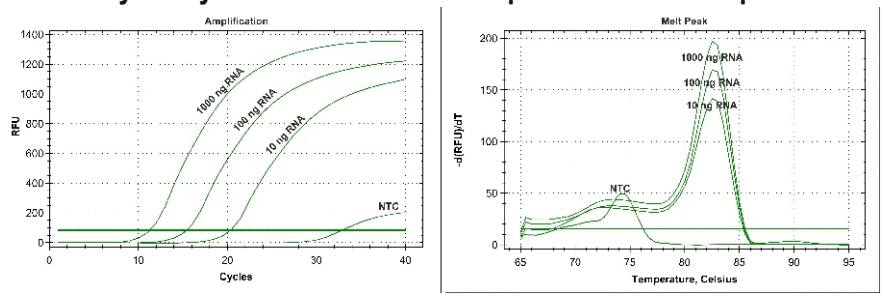
* Depends on the primer Tm and template used (Need to be optimized)

** Allow approximately 1 minutes for every 1kb to be amplified.

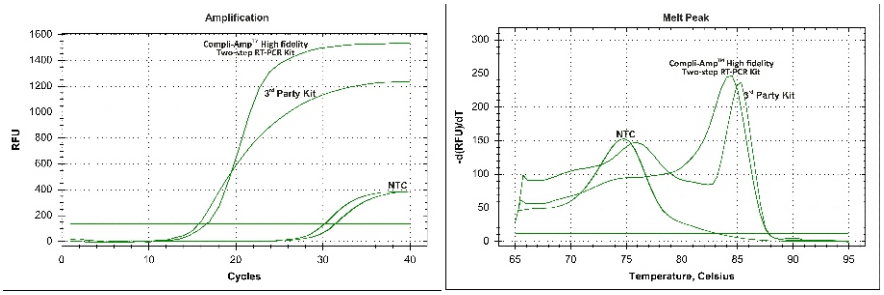
Note: *The above condition is optimized based on BioRAD CFX Maestro Dx SE. The kit works best with another qPCR machine also. Keep the PCR condition as optimized for SyberGreen.*

Result

Sensitivity assay for the amount of RNA present in the sample



Bench marking of Compli-Amp™ High fidelity Two-step RT-PCR Kit with 3rd Party kit



Note: The melting curve scale of NTC should be sifted when compared with the targeted amplicon.

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	Maintain aseptic conditions; add RNase inhibitor. (Although Buffer already contains inhibitor)
	Not enough starting template RNA	Increase the concentration of template RNA; use 100 ng to 1µg 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 potentially 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