Compli-Amp™ Green One-Step RT-PCR Kit

PRODUCT DETAILS	STORAGE & HANDLING
Product: Compli-Amp™ Green One-Step RT-PCR Kit Catalogue: RNA011-50 (50 reactions); RNA011-100 (100 reactions); SP-RNA011-50 (Sample Pack for 5 reactions)	Storage: −20°C Keep reaction components and samples on ice during setup. Transfer reactions promptly to a preheated thermal cycler.

Introduction

The Compli-Amp Green One-Step RT-PCR Kit performs cDNA synthesis and PCR amplification in a single tube using gene-specific primers, starting from total RNA or mRNA. It combines MMLV Reverse Transcriptase and Taq DNA Polymerase in an optimized reaction buffer, with GreenR™ nucleic-acid dye in the master mix for dye-based RT-PCR. The kit supports RNA targets from 100 bp to more than 6 kb and uses 20 ng to 1 µg total RNA as starting material. Single-tube processing reduces reagent-handling steps and the risk of contamination.

The engineered MMLV Reverse Transcriptase has reduced RNase H activity and improved thermal stability. cDNA synthesis at 42–50°C supports structured RNA templates and full-length cDNA generation.

Kit Contents

Cat. No.	Component	50 Rxn	100 Rxn	5 Rxn
RNA-100	Enzyme mix	25 µL	50 µL	2.5 µL
RNA-200	2X Standard PCR Master Mix with GreenR	500 µL	1000 µL	50 µL
RNA-1400	100 mM DTT	50 µL	100 µL	5 µL

Highlights

One-tube workflow: Reverse transcription and PCR amplification without separate reagent additions.

Broad target range: Designed for RNA targets from 100 bp to >6 kb.

Integrated GreenR dye: Suitable for dye-based RT-PCR detection.

Thermostable RT: Engineered MMLV enzyme supports cDNA synthesis at 42–50°C.

Guidelines for RNA Quality and Quantity

Use high-quality, intact, RNase-free RNA. For low-copy-number genes or longer targets, use more starting material (>100 ng total RNA). Assess RNA integrity by Bioanalyser and gel electrophoresis. Maintain aseptic conditions during isolation; TRIzol-type or spin-column RNA isolation methods may be used.

Reaction Setup — 20 µL Total Volume

Component	Amount
2X Standard PCR Master Mix	10 µL
Enzyme mix (RNA-100)	0.5 µL
Forward primer	0.5 µL of 10 µM
Reverse primer	0.5 µL of 10 µM
RNA template (10 ng–1 µg)	Variable
Nuclease-free water (NFW)	Variable
Total	20 µL

Preparation: Preheat the thermal cycler to 42–50°C. Keep all components, mixes, and samples on ice. After setup, transfer tubes to the preheated cycler and start the RT-PCR program immediately. cDNA synthesis is typically carried out for 15–30 minutes at 42–50°C.

Thermal Cycling Conditions

Step	Temperature	Time / cycles
cDNA synthesis	42°C	30 min
RT inactivation	95°C	10 min
Denaturation	94°C	15 sec × 35 cycles
Annealing	55–65°C*	30 sec × 35 cycles
Extension	72°C	1 min/kb × 35 cycles
Final extension (optional)	72°C	5 min

*The optimal reverse-transcription temperature and cycling conditions depend on primer and target sequences; optimize for the specific assay.

Gel Loading Protocol

1. Mix the DNA sample with GreenTrack 6X Loading Dye as directed in the source protocol.
2. Load the sample onto an agarose gel and perform electrophoresis.
3. Monitor DNA migration and visualize the products using an appropriate staining method.

Representative RT-PCR Results

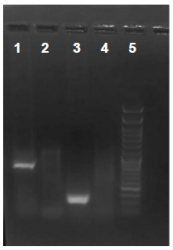

Figure One step RT-PCR of a 180 bp and 400 bp gene fragment with 100ng of total RNA (Human Blood) using β-actin and GAPDH primers.
Lane 1 - 400 bp PCR product using GAPDH primers
Lane 2 - Negative control (NC).
Lane 3 - 180 bp PCR product using β-Actin primers
Lane 4 - NC
Lane 5 - 1kb ladder

Figure 1. One-step RT-PCR of 180 bp and 400 bp gene fragments using 100 ng total RNA (human blood) with β-actin and GAPDH primers. Lane 1: 400 bp GAPDH PCR product; Lane 2: Negative control; Lane 3: 180 bp β-actin PCR product; Lane 4: Negative control; Lane 5: 1 kb ladder.

Troubleshooting

Observation	Possible cause	Recommended action
No amplification product	RT temperature too high	Lower the reverse-transcription temperature.
No amplification product	RNase contamination	Maintain aseptic conditions; consider an RNase inhibitor.
No amplification product	Insufficient or degraded RNA	Increase template RNA (100 ng–1 µg) or replace degraded RNA.
No amplification product	RT inhibitors in RNA	Remove inhibitors; an additional 70% ethanol wash may help.
No amplification product	Annealing temperature too high / extension too short / too few cycles	Reduce annealing temperature; allow 60 sec/kb extension; increase cycle number.
Low specificity	Suboptimal reaction conditions	Optimize Mg^{2+}, primers, annealing temperature, extension time and RT temperature.
Low specificity	Oligo(dT) or random primers	Use gene-specific primers.
Unexpected bands	Genomic DNA contamination	DNase I-treat RNA; design exon-spanning or exon–exon junction primers.
Unexpected bands	Nonspecific primer annealing	Adjust annealing temperature and magnesium concentration.
Unexpected bands	Primer dimers	Redesign primers to avoid 3′ complementarity.

Technical note: The source datasheet lists primer input in µM rather than µL, and its gel-loading dye ratio is described inconsistently with the designation “6X”. Verify these details against the validated laboratory protocol before use.