For research use only,
not for use in diagnostic procedures.

iNtRON Biotechnology

Instruction for Use

RealMOD™ Green T²
2X qRT-PCR mix

version; 1.0
Jun. 2026

Cat. No.	**25365.100**	**100 Tests**
Cat. No.	**25365.300**	**300 Tests**
		for 20 µl reactions

Store the Kit at –20°C or below

www.intronbio.com

[INDEX]

1. DESCRIPTION

RealMOD™ Green T² 2X qRT-PCR mix is a premium, dye-based, one-step qRT-PCR master mix engineered for high-sensitivity and high-throughput quantitative RNA detection. Conventional dye-based assays frequently encounter critical technical limitations, such as sample dropouts in low-concentration targets and background interference caused by template-independent non-specific amplification. This advanced formulation comprehensively addresses these challenges by integrating an antibody-mediated hot-start Taq DNA Polymerase with a highly optimized, temperature-activated Reverse Transcriptase within a proprietary buffer infrastructure, maximizing both reaction sensitivity and specificity.

To guarantee absolute data integrity and eliminate false-positive results, the kit incorporates a specialized 5× gDNA Eraser that rapidly and enzymatically eliminates contaminating host genomic DNA from RNA templates without compromising transcript stability. Supplied as a convenient 2× Master Mix pre-blended with a non-interfering visual tracking dye for immediate visual verification of pipetting accuracy and a universal ROX reference dye, this stabilized matrix guarantees seamless, calibration-free compatibility across all qPCR platforms, ensuring high-performance amplification with only the addition of target-specific primers and template.

2. FEATURES

- Visual Co-Tracking Assurance: Integrates a proprietary, non-interfering visual tracking dye that enables effortless, real-time monitoring of multi-well pipetting trajectories without compromising quantitative fluorescence kinetics.
- Rapid Enzymatic gDNA Clearance: Outfitted with the high-efficiency 5× gDNA Eraser to actively degrade contaminating host genomic architecture within 2 minutes, ensuring template purity prior to amplification.
- Stringent Amplification Specificity: Features an advanced dual-lock hot-start system —combining an antibody-mediated Taq DNA Polymerase and a temperature-activated Reverse Transcriptase—to rigorously suppress primer-dimers and non-specific artifacts.
- Robust Carryover Contamination Control: Incorporates an active dUTP/UDG decontamination matrix that enzymatically destroys ambient aerosol amplicon contaminants at room temperature to preserve absolute data integrity.
- Universal Platform Integration: Pre-blended with a optimized universal ROX reference dye, enabling calibration-free, high-performance execution across both high-ROX and low-ROX qPCR instrumentation scales.

3. KIT CONTENTS

Components	25365.100 (100 rxns)	25365.300 (300 rxns)	Core Composition & Functional Profile
RealMOD™ Green T² 2X qRT-PCR mix	1.0 ml × 1 vial	1.0 ml × 3 vials	Consists of Hot-Start Taq, temperature-activated RTase, SYBR Green I, Universal ROX reference dye, non-interfering visual tracking dye matrix, dNTPs/dUTP mix, and stabilizing reaction buffer.
5× gDNA Eraser	400 µl × 1 vial	400 µl × 3 vials	Contains a recombinant, sequence-specific endonuclease optimized for rapid enzymatic degradation of background genomic DNA contaminants.
DNase/RNase-Free Water	1.0 ml × 1 vial	1.0 ml × 3 vials	Ultrapure, nuclease-free liquid substrate.

4. STORAGE CONDITIONS

- **Temperature Regimen** : Store strictly **at -20°C or below** in a constant-temperature, non-frost-free laboratory freezer.
- **Photostability Management : Crucial.** Protect from direct light at all times. Prolonged exposure to ambient illumination will severely compromise the fluorescence quantum yield of SYBR Green I and ROX reference dyes, resulting in signal attenuation.
- **Thermal Shipping Specification** : Dispatched via validated dry ice configuration (≤ 0°C). Verify that the core container matrix has remained completely frozen upon arrival.
- **Stability & Maintenance** : Stable for up to 12 months from the date of manufacture under recommended storage parameters. To ensure consistent enzymatic kinetics, avoid excessive freeze-thaw cycles. Aliquoting is recommended if the kit is frozen and thawed frequently during routine operations.

5. BEFORE STARTING

1) General Precautions

- Nuclease-Free Environment: RNA is highly susceptible to enzymatic degradation. Prior to initiation, decontaminate all workspaces, pipettes, and equipment using validated RNase decontamination solutions. Standard protective gear, including disposable gloves and masks, must be worn throughout the entire procedure.
- Thermal Management: Maintain all functional kit components on an ice bath or cold block during reaction assembly. Avoid prolonged exposure of the master mix to ambient room temperature to preserve baseline enzyme kinetics.
- Visual Verification: Ensure all frozen reagents are completely thawed and exhibit no visible precipitation or phase separation before use.

2) Materials Required but Not Provided

- Real-Time PCR Instrumentation: Fully calibrated qPCR thermal cyclers capable of detecting SYBR Green I (FAM channel) and processing ROX reference signals.
- Nuclease-Free Consumables: Certified RNase-free and DNase-free microcentrifuge tubes, and high-optical-clarity PCR tubes or multi-well plates with compatible optical adhesive seals.
- Liquid Handling Tools: Dedicated high-precision micropipettes equipped with sterile, certified aerosol-barrier (filter) nuclease-free pipette tips.
- Centrifugation Equipment: Microcentrifuge and dedicated plate centrifuge capable of displacing ambient air bubbles from analytical wells.
- Thermal Incubator: Standard laboratory heating block or water bath calibrated to maintain 42℃ for the genomic DNA clearance stage.

3) Crucial Handling Notes

- Complete Homogenization: Thoroughly thaw the RealMOD™ Green T² 2X qRT-PCR mix on ice. Prior to opening, vigorously vortex the vial for 5 seconds and spin down briefly. This step is imperative to fully homogenize the unique visual tracking dye matrix and highly concentrated enzyme complexes, preventing well-to-well Ct variation.
- Template Input Quantification: Dilute the template RNA so that a volume of 2–5 µl is administered per 20 µl reaction. To avoid extraction-derived reaction inhibitors and ensure a precise linear dynamic range, adhere strictly to the following mass input parameters:
  - Total RNA: 10 pg to 1 µg per reaction
  - mRNA: 10 fg to 100 ng per reaction
- Instrument ROX Normalization: This formulation incorporates a universal ROX reference dye optimized to automatically calibrate optical variance across diverse qPCR platforms. Ensure that the instrument's internal reference channel (Passive Reference) is configured to 'ROX' to utilize the real-time baseline normalization function effectively.

6. EXPERIMENTAL PROTOCOL

6.1. Genomic DNA Removal from RNA Template (Optional)

This pre-treatment sequence eliminates background genomic DNA from the isolated RNA template prior to the main amplification. If genomic removal is not required, omit this section and proceed directly to Section 6.2.

1) Dispense the reaction components into a sterile, RNase-free microcentrifuge tube on ice according to the specifications detailed in the grid below:

- 5× gDNA Eraser: 4.0 µl
- Template RNA: Total RNA: 1 pg – 1 µg
- DNase/RNase-Free Water : to 20.0 µl

2) Mix the solution gently by pipetting up and down or briefly vortexing, and then spin down the tube to collect the liquid at the bottom.

3) Incubate the mixture at 42°C for 2 minutes using a calibrated heating block or water bath.

- **Note :** Do not premix this pre-treatment component with primers prior to this thermal incubation step.

6.2 Preparation and Execution of qRT-PCR Main Reaction

1) Arrange new RNase-free microcentrifuge tubes or a qPCR multi-well plate on an ice block to maintain low-temperature stability.

2) Add each baseline component precisely into the reaction vessels according to the specific volumes detailed in Table 1 :

Table 1. Reaction Mixture Configuration

Components	Volume
RealMOD™ Green T² 2X qRT-PCR mix	10.0 µl
Primer Forward (10 µM)	0.4 µl
Primer Reverse (10 µM)	0.4 µl
Template RNA (from Section 6.1 or crude RNA)	Total RNA: 1 pg – 1 µg
DNase/RNase-Free Water	to 20.0 µl
Total Volume Matrix	20.0 µl

- **Note :** If utilizing the gDNA Eraser pre-treated matrix from Section 6.1, adjust the template aliquot volume within a range of 1.0 – 9.2 µl based on template concentration to ensure the final absolute mass input complies with the validated

dynamic range (1 pg – 1 µg). The recommended optimal addition volume is 2.0 – 5.0 µl.

- **Note :** Optimize individual primer parameters within a range of 0.1 – 1.0 µM only if the baseline configuration (0.2 µM final concentration) yields suboptimal kinetic efficiency. Keep primer inputs minimized to suppress non-specific background artifacts.

3) Homogenize the complete mixture gently by meticulous pipetting or controlled vortexing, and spin down the tubes or plate immediately to displace ambient air bubbles from the analytical wells.

4) Program your real-time PCR instrumentation by selecting either the Standard Protocol (Table 2) or the Fast Protocol (Table 3) based on your operational throughput requirements, and execute the run immediately.

Table 2. Standard Program Configuration

Program Step	Temperature	Time	Cycles
Reverse Transcription	50–55°C	5 min*	1
Initial Denaturation	95°C	30 sec	1
Denaturation	95°C	10 sec	40
Annealing / Extension[1]	60°C	30 sec	
Melt Curve	Instrument Default	Instrument Default	1

* Can be extended to 15 min for low-copy targets.
[1] Set real-time fluorescence signal acquisition at this step.

Table 3. Fast Program Configuration (Select "Fast" mode under Run Mode)

Program Step	Temperature	Time	Cycles
Reverse Transcription	50–55°C	3 min*	1
Initial Denaturation	95°C	30 sec	1
Denaturation	95°C	5 sec	40
Annealing / Extension[1]	60°C	20 sec	
Melt Curve	Instrument Default	Instrument Default	1

[1] Set real-time fluorescence signal acquisition at this step.

5) Verify the mathematical validity of the data upon thermal cycle completion by evaluating the exponential amplification curve and melting curve profiles.

6) Generate the standard calibration curve utilizing known concentration plots to execute high-precision target quantification analysis.

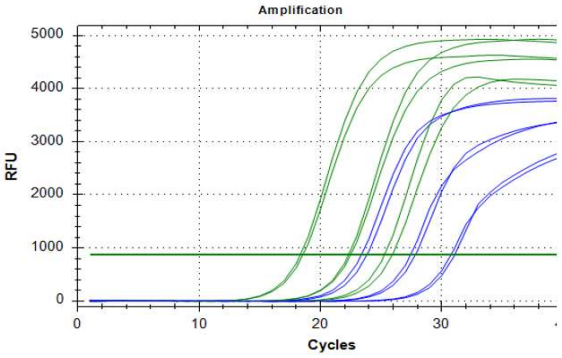
7. EXPERIMENTAL DATA (ANALYSIS)

1) Superior Amplification Efficiency, High Sensitivity, and Broad Versatility

- RealMOD™ Green T² 2X qRT-PCR mix delivers unmatched amplification efficiency, exceptional sensitivity, and reliable performance across diverse RNA templates, including both cellular and viral targets.

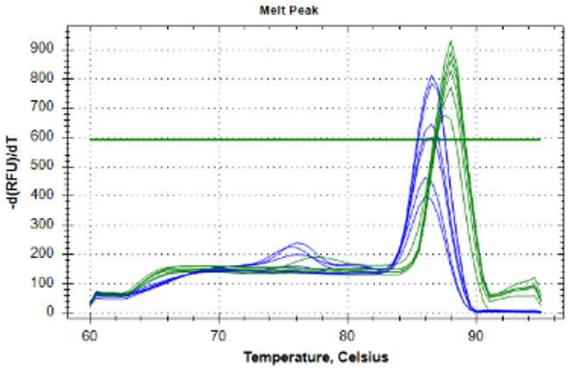
Figure 1. Performance Comparison against Competitor (GAPDH Target)

(A) Amplification Plot (FAM Channel)



RealMOD™ Green T² : Green color
Supplier A : Blue color curve

(B) Melting Curve Profile



RealMOD™ Green T² : Green color
Supplier A : Blue color curve

- Experimental Assay Criteria: Amplification curves and melt peaks of the house-keeping GAPDH gene from K562 cellular total RNA.
- Dilution Matrix Parameters: The RNA template was 10-fold serially diluted from 20 ng down to 200 pg (executed in duplicate).
- Performance Validation: The RealMOD™ Green T² 2X qRT-PCR mix demonstrates significantly earlier Ct values, higher final fluorescence intensities, and specific single-target amplification profile compared to Supplier A.

2) Ultra-Low Mass High-Sensitivity Viral RNA Detection

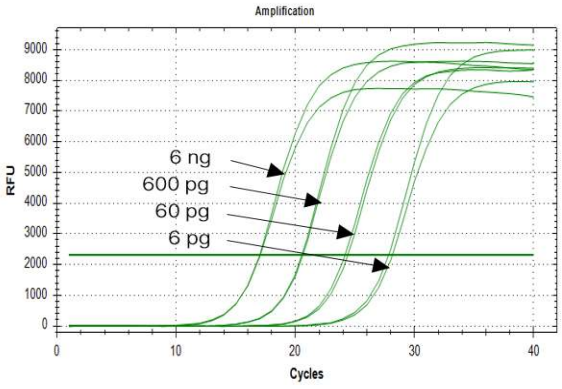


Figure 2. Linear Dynamic Range for Viral Copy Targets (CPIV RNA)

Verification of the broad linear dynamic range and high-sensitivity quantification matrix utilizing 10-fold serially diluted CPIV viral RNA samples (6 ng down to 6 pg) in duplicate amplification kinetics.

- Experimental Assay Criteria: Amplification curves of viral CPIV RNA targets.
- Dilution Matrix Parameters: The viral template architecture was 10-fold serially diluted from 6 ng down to 6 pg (executed in duplicate).
- Performance Validation: The evenly spaced amplification curves demonstrate excellent amplification efficiency and highly sensitive detection capability even at ultra-low RNA concentration thresholds, validating the kit's broad compatibility and performance stability with diverse, low-copy RNA types.

8. TROUBLESHOOTING GUIDE

Symptom	Possible Cause	Recommendation
No Amplification Curve or Delayed Ct Values (Little to no product)	Pipetting error or missing reagent	Verify reaction setup, individual component volumes, and expiration dates before repeating the assay.
	RNA template degraded or insufficient amount	Use high-quality, intact RNA and certified RNase-free consumables. Avoid repetitive freeze-thaw cycles and increase the baseline RNA input mass.
	Primer concentration not optimal or degraded	Optimize final primer concentration parameters within the range of 0.2 – 0.4 µM. Use freshly synthesized aliquots if degradation is suspected.
	Suboptimal cycling conditions (RT or PCR steps)	Verify reverse transcription (RT) and initial denaturation (Hot-Start) temperatures and hold times against the standard specifications detailed in Section 6.2.
	Presence of qRT-PCR inhibitors in the template	Dilute the RNA template matrix (1:10 or 1:100) and repeat the amplification to minimize chemical downstream inhibitor carryover effects.
	High RNA secondary structure or high GC content	Extend the RT reaction duration up to 15 minutes or optimize the

Symptom	Possible Cause	Recommendation
		RT temperature parameters to facilitate efficient melting of complex secondary structures.
	Insufficient number of cycles	Increase the total programmed thermal cycling threshold to at least 40 cycles.
Multiple Melt Peaks, Primer-Dimers, or Amplification in NTC	Contamination of reagents or workspace	Replace current master mix and reagent aliquots with fresh stocks. Use aerosol-barrier filter tips and thoroughly decontaminate the designated molecular biology workstation.
	Primer design not optimal	Redesign assay primers to evaluate exact Tm profiles, avoid self-complementarity infrastructure, and prevent non-specific primer-dimer artifacts.
	Annealing temperature too low	Increase the programmed annealing temperature increments by 1°C to 2°C to enhance highly stringent binding kinetics.
	Primer concentration too high	Decrease the final primer concentration criteria down to a clean range of 0.1 – 0.2 µM to suppress non-specific background signals.
Poor Amplification Efficiency (Non-linear standard curve or Low R^2 value)	Inaccurate pipetting of serial dilutions	Use calibrated high-precision pipettes and mix thoroughly prior to executing each subsequent serial dilution step to ensure strict linear homogeneity.
	Amplification inhibition at high template concentrations	Discard the highest template concentration data point or further dilute the starting sample template architecture before loading the plate.

9. ORDERING INFORMATION

Product	Cat. No.	Q'ty
RealMOD™ Green T² 2X qRT-PCR mix	25365	100 rxns / 300 rxns
RealMOD™ Green T² 2X qPCR mix	25364	200 rxns / 500 rxns
RealMOD™ Probe M² 2X qRT-PCR mix (with UDG)	25361	100 rxns / 500 rxns
easy-spin™ Total RNA Extraction Kit	17221	50 col.
RNA-spin™ Total RNA Extraction Kit	17211	50 col.
Viral Gene-spin™ Viral DNA/RNA Extraction Kit	17151	50 col.
IQeasy™ plus Plant RNA Extraction Kit	17491	50 col.

iNtRON Biotechnology, Inc.
701~ 704. Jung-AngInduspiaV,
Sangdaewon-dong, 137, Sagimakgol-ro,
Joongwon-gu, Sungnam-si, Gyeonggi-do,
Korea

(Review Date : 2026. 6. 18)