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

iNtRON Biotechnology

Instruction for Use

RealMOD™ Green T²
2X qPCR mix

version; 1.0
Jun. 2026

Cat. No.	**25364.200**	**200 Tests**
Cat. No.	**25364.500**	**500 Tests**
		for 20 µl reactions

Store the Kit at –20°C or below

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1. DESCRIPTION

RealMOD™ Green T² 2X qPCR mix is a specialized premix designed for highly sensitive quantitative PCR using the SYBR Green I chimeric fluorescence method. The core component features a next-generation, antibody-modified hot-start Taq DNA Polymerase, which ensures robust specificity, high detection sensitivity, and superior amplification yield.

Combined with a specially optimized qPCR buffer system and a specific enhancer, this product delivers exceptional performance for the amplification and quantification of various DNA samples, including genomic DNA, cDNA, and plasmid DNA. Supplied as a convenient 2× master mix, it requires only the addition of primers and a template. Furthermore, it includes a Specific ROX Reference Dye, making it universally compatible with all qPCR instruments without any need for ROX concentration adjustments.

2. FEATURES

- Ready-to-use formulation: A convenient 2× master mix requiring only the addition of primers and template for amplification.
- High specificity and yield: Powered by a next-generation hot-start Taq DNA Polymerase modified via an antibody method to ensure high detection sensitivity.
- Universal instrument compatibility: Contains a Specific ROX Reference Dye suitable for all qPCR platforms without requiring ROX concentration adjustments.
- Optimized performance: Includes an optimized buffer system and specific enhancers to maximize specificity and sensitivity.

3. KIT CONTENTS

Components	25364.200 (200 rxns)	25364.500 (500 rxns)	Core Composition & Functional Profile
RealMOD™ Green T² 2X qPCR mix	1.0 ml × 2 vials	1.0 ml × 5 vials	Consists of next-generation antibody-modified hot-start Taq DNA Polymerase, SYBR Green I chimeric fluorescent dye, Specific ROX Reference Dye, dNTPs mix, Mg^{2+}, and optimized qPCR reaction buffer substrate.
DNase/RNase-free Water	1.0 ml × 2 vials	1.0 ml × 5 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

- Contamination Prevention: Due to the remarkably high detection sensitivity of this kit, reactions are easily contaminated by aerosols in the air. The preparation of the reaction system should strictly be carried out in a clean bench. Standard protective gear must be worn, and a sterile, DNase/RNase-free environment must be maintained throughout the procedure.
- Thermal & Light Management: Maintain kit components on an ice bath or cold block during reaction assembly. Because the master mix contains the fluorescent dye SYBR Green I, avoid strong light exposure during preparation to preserve signal integrity.
- Visual Verification & Handling: Ensure all frozen reagents are thawed completely. If a white precipitate is found in the Master Mix after thawing, place it at room temperature for a short while and invert it upside down several times to fully dissolve the precipitate 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.
- Sterile Consumables: Certified DNase-free and RNase-free sterile reaction tubes or multi-well plates with compatible optical adhesive seals.
- Liquid Handling Tools: Dedicated high-precision micropipettes equipped with sterile, certified aerosol-barrier(filter) pipette tips to prevent aerosol contamination.
- Centrifugation Equipment: Microcentrifuge and dedicated plate centrifuge capable of displacing ambient air bubbles from analytical wells, as air bubbles will affect the quantitative results.

3) Crucial Handling Notes

- Complete Homogenization: Thoroughly thaw the RealMOD™ Green T² 2X qPCR mix. If a white precipitate is found after thawing, place it at room temperature for a short while and invert it upside down several times to dissolve the precipitate before use. Do not vortex to avoid air bubbles, which will negatively affect the quantitative results. Spin down briefly to collect the contents at the bottom.
- Template Input Quantification: Due to the high sensitivity of qPCR, dilute the template DNA (e.g., cDNA, genomic DNA, plasmid DNA) and add it to the system to improve the repeatability of the experiment. The volume of undiluted cDNA template should be ≤ 1/10 of the total reaction volume. Furthermore, the formulation's exceptional sensitivity ensures robust performance even with trace amounts of target, successfully amplifying and detecting as low as 6 copies of plasmid DNA (e.g., pUC19) per reaction.
- Instrument ROX Normalization: This kit contains a Specific ROX Reference Dye, which is suitable for all qPCR instruments without adjusting the concentration of ROX on different instruments. 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

1) Arrange new sterile, DNase/RNase-free reaction tubes or a qPCR multi-well plate on an ice block or cold bath to maintain low-temperature stability during reaction assembly.

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
2× RealMOD™ Green T² qPCR Master Mix	10.0 µl
Primer Forward (10 µM)	0.4 µl
Primer Reverse (10 µM)	0.4 µl
Template DNA / cDNA	variable
DNase/RNase-free Water	to 20.0 µl
Total Volume Matrix	20.0 µl

- **Note :** The final concentration of the primers is generally recommended to be 0.2 µM to obtain optimal amplification efficacy. Optimize individual primer parameters within a range of 0.1 – 1.0 µM only if the baseline configuration yields suboptimal kinetic efficiency. Keep primer inputs minimized to suppress non-specific background artifacts.
- **Note :** Due to the high sensitivity of the qPCR system, dilute the template prior to addition to improve experimental repeatability. Ensure the volume of undiluted cDNA template is ≤ 1/10 of the total reaction volume. The formulation's exceptional sensitivity ensures robust performance even with trace amounts of target, successfully amplifying and detecting as low as 6 copies of plasmid DNA (e.g., pUC19) per reaction.
- **Note :** Mix the complete reaction solution thoroughly by inverting the tube several times. Carefully avoid vortexing to prevent the formation of ambient air bubbles that could negatively affect quantitative optical results, and follow immediately with a brief centrifugation to collect the contents at the bottom of the vessel.

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

4) Program your real-time PCR instrumentation according to the conditions detailed in Table 2, and execute the run.

Table 2. qPCR Program Configuration

Program Step	Temperature	Time	Cycles
Initial Denaturation	95°C	30 sec	1
Denaturation	95°C	3-10 sec	40
Annealing / Extension[1]	60°C	10-30 sec	
Melt Curve	Instrument Default	Instrument Default	1

[1] Set real-time fluorescence signal acquisition at this step.
Set the extension time to 10 seconds for amplicons within 200 bp. For amplicons exceeding 200 bp, an extension time of 30 seconds is recommended to guarantee complete synthesis yield.

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.**

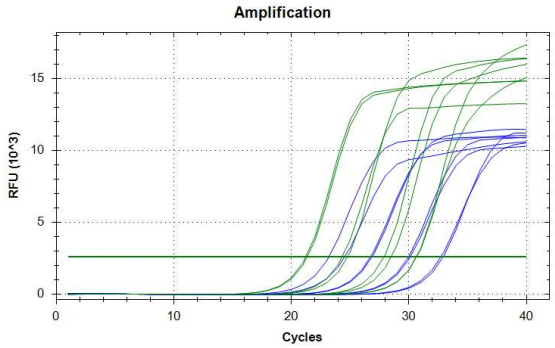
7. EXPERIMENTAL DATA (ANALYSIS)

1) Superior Amplification Efficiency, High Sensitivity, and Yield

- RealMOD™ Green T² 2X qPCR mix delivers unmatched amplification efficiency, exceptional sensitivity, and robust performance across diverse DNA templates, ensuring precise quantitative analysis.

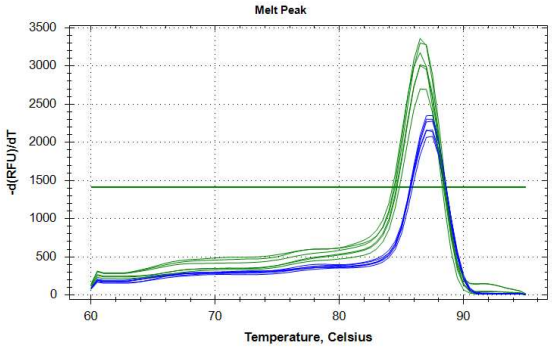
Figure 1. Performance Comparison against Competitor (GAPDH Target)

(A) Amplification Plot (FAM Channel)



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

(B) Melting Curve Profile



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

- Experimental Assay Criteria: Amplification curves and melt peaks of the house-keeping GAPDH gene amplified from K562 genomic DNA (gDNA).

- Dilution Matrix Parameters: The K562 gDNA template was 10-fold serially diluted from 5 ng down to 5 pg.
- Performance Validation: The RealMOD™ Green T² 2X qPCR mix demonstrates significantly earlier Ct values and higher final fluorescence intensities compared to Supplier A, indicating superior detection sensitivity and amplification yield. Furthermore, the sharp, singular melting peak confirms stringent target specificity without non-specific background artifacts.

2) Broad Template Versatility: Robust cDNA Amplification

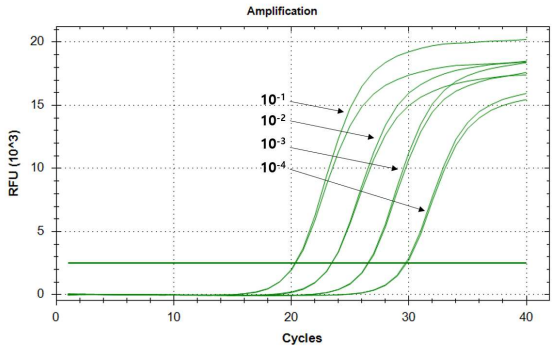


Figure 2. Linear Dynamic Range across cDNA Dilution Series

Verification of the broad linear dynamic range and high-sensitivity quantification matrix utilizing 10-fold serially diluted cDNA templates (10^{-1} down to 10^{-4}) in duplicate amplification kinetics.

- Experimental Assay Criteria: Following the successful gDNA amplification demonstrated in Figure 1, this assay evaluates the master mix's performance utilizing a cDNA template. The cDNA was synthesized utilizing the Maxime™ RT PreMix (Random Primer) using CPIV RNA and subsequently amplified.
- Dilution Matrix Parameters: The synthesized cDNA template was 10-fold serially diluted from 10^{-1} down to 10^{-4}, as indicated by the arrows in the amplification plot.
- Performance Validation: The uniformly spaced amplification curves across the specific dilution points (10^{-1} to 10^{-4}) demonstrate exceptional linearity and high amplification efficiency. This validates the kit's broad template compatibility, proving it delivers highly reliable and stable quantitative performance whether utilizing complex genomic DNA or synthesized cDNA templates.

8. TROUBLESHOOTING GUIDE

Symptom	Possible Cause	Recommendation
No Amplification Curve or Delayed Ct Values (Little to no product)	Insufficient number of cycles	Set the number of cycles to 40. Too many cycles will increase background signals and reduce data reliability.
	Signal acquisition step not set	Confirm whether the signal acquisition step is set up in the program. Set the signal acquisition at the annealing and extension stage.
	Template degraded, low concentration, or contains inhibitors	Prepare new template and retry. If concentration is low, reduce the dilution factor. If inhibitors are present, increase the dilution factor.
	Primer issues or long PCR products	Confirm whether primers are degraded and test integrity. Ensure the recommended length of PCR products is 80–150 bp. Redesign primers if amplification efficiency is low.
	Custom Primer Tm Match	If custom primers fail at 60°C, optimize annealing down to 55°C.
Abnormal Shape of Amplification Plot	Bubbles remaining in the reaction tube	Pay attention to centrifugation when processing samples and carefully check the reaction tube for any remaining bubbles before performing reaction.
	Template concentration too high	If the baseline endpoint value is greater than the Ct value (broken/downward plot), reduce the baseline endpoint (Ct value - 4) and repeat data analysis.
	Signal is too weak	Increase template concentration and retry if the plot is rough.

Symptom	Possible Cause	Recommendation
Multiple Peaks in the Melting Curve	Inappropriate primer design or concentration	Design and synthesize new primers according to primer design principles. Decrease the primer concentration if it is too high. Increase annealing temperature by 1-2°C.
	cDNA template contaminated with genomic DNA	Prepare new cDNA templates to eliminate genomic DNA contamination.
Amplification Observed in Negative Control (NTC)	Contamination of reaction system	Replace with new mix, ddH_2O, and primers to repeat the experiment. Prepare the reaction system in a clean bench to reduce aerosol contamination.
	Primer dimer formation	Carry out analysis in association with the melting curve.
Poor Experiment Repeatability or Non-linear Standard Curve	Deviations of pipetting volume	Use a higher performance pipette. Increase the dilution factor of the template and increase the pipetting volume accordingly to minimize errors.
	Low template concentration	The lower the template concentration, the worse the repeatability. Reduce the template dilution factor or increase the volume of sample addition.
	Differences in instrument temperature control	Calibrate the qPCR instrument regularly to ensure uniform temperature across all wells.

9. ORDERING INFORMATION

Product	Cat. No.	Q'ty
RealMOD™ Green T² 2X qRT-PCR mix (with UDG)	25365	100 rxn / 300 rxn
RealMOD™ Probe M² 2X qRT-PCR mix (with UDG)	25361	100 rxn / 500 rxn
G-spin™ Total DNA Extraction Mini Kit	17045 / 17046	50 col. / 200 col.
DNA-spin™ Plasmid DNA Purification Kit	17096 / 17098	50 col. / 200 col.
Viral Gene-spin™ Viral DNA/RNA Extraction Kit	17151	50 col.
Maxime™ RT PreMix (Oligo (dT)15 Primer)	25081	96 tubes
Maxime™ RT PreMix (Random Primer)	25082	96 tubes

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