RealMOD™ Green T² 2X qRT-PCR Mix

QUICK GUIDE

100 Tests : 25365.100
300 Tests : 25365.300

1. KIT DESCRIPTION

RealMOD™ Green T² 2X qRT-PCR mix is a premium one-step master mix featuring an antibody-mediated hot-start Taq and temperature-activated RTase dual-lock system to strictly suppress non-specific amplification. The kit includes a high-efficiency 5x gDNA Eraser that clears contaminating background genomic DNA within 2 minutes , alongside an active dUTP/UDG matrix for robust carryover contamination control. Pre-blended with a universal ROX reference dye for seamless platform compatibility and a non-interfering visual tracking blue dye to ensure pipetting accuracy , this optimized formulation delivers high-sensitivity, calibration-free quantitative RNA detection.

2. KIT CONTENTS AND STORAGE

Contents	100 rxns	300 rxns
RealMOD™ Green T² 2X qRT-PCR mix	1.0 ml	1.0 ml x 3
5x gDNA Eraser	400 µl	400 µl x 3
Nuclease-free Water	1.0 ml	1.0 ml x 3

- Storage Conditions: Store strictly at ≤ -20ºC
- Protect from direct light at all times and avoid excessive freeze-thaw cycles.

3. GENOMIC DNA REMOVAL FROM RNA (Optional)

1) Dispense the reaction matrix into a sterile, RNase-free tube on ice ; 5x gDNA Eraser - 4.0 µl | RNA (Total RNA 1 pg–1 µg) | RNase-free Water to 20.0 µl
2) Mix the solution gently by pipetting or brief vortexing, and spin down to collect the liquid substrate.
3) Incubate the mixture at 42°C for 2 minutes using a calibrated heating block or water bath.
4) Transfer immediately to an ice-water bath to rapidly cool and stabilize the processed template.

NOTE: Do not premix this component with target primers prior to this step.

4. PREPARATION OF RT-qPCR REACTION MIXTURE

Components	Volume	Notes
RealMOD™ Green T² 2X qRT-PCR mix	10.0 µl	Includes Hot-Start Taq & active RTase.
Primers (F / R, 10 µM)	0.4 µl / 0.4 µl	Default final concentration is 0.2 µM.
Nuclease-free Water	to 20.0 µl	Ultrapure liquid substrate.
Template RNA	Variable	Add 2.0 – 5.0 µl optimal volume (1 pg – 1 µg) .
Total Volume	20.0 µl	Mix gently, spin down, and run immediately.

5. INSTRUMENTATION PROGRAM SELECTION

Program	Temp.	Mode		Cycles
		Standard	Fast	
R.T.Reaction	50~55 ºC	5 min*	3 min	1
Predenaturation	95 ºC	30 sec	30 sec	1
Denaturation	95 ºC	10 sec	5 sec	40
Ann./Extension[1]	60 ºC	30 sec	20 sec	
Melt Curve		Instrument Default		1

* Standard RT can be extended to 15 min for ultra-low copy targets.
[1] Configure your qPCR software to acquire real-time fluorescence signals (FAM channel) at this step.

6. TROUBLESHOOTING

Symptom	Possible Cause	Immediate Recommendation
No Amplification Curve or Delayed Ct Values	• Pipetting / Reagent error • Template degradation • qRT-PCR Inhibitors • Custom Primer Tm Match	• Verify individual volumes and expiration dates before repeating. • Use high-quality RNA; avoid repeated freeze-thaw cycles. • Dilute template (1:10 or 1:100) to bypass PCR inhibitors. • If custom primers fail at 60°C, optimize annealing down to 55°C.
Multiple Melt Peaks, Primer-Dimers, or NTC Signal	• Workspace contamination • Primer design defect • Annealing temp too low • Primer input too high	• Replace mix aliquots, use aerosol filter tips, and decontaminate bench. • Redesign primers to ensure appropriate Tm and avoid self-complementarity. • Increase annealing temperature by 1–2°C. • Decrease final primer concentration to 0.1–0.2 µM.