RealMOD™ Green T² 2X qPCR Mix

QUICK GUIDE

200 Tests : 25364.200
500 Tests : 25364.500

1. KIT 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. Featuring a next-generation, antibody-modified hot-start Taq DNA Polymerase, it ensures robust specificity and high detection sensitivity. Pre-blended with a Specific ROX Reference Dye for universal platform compatibility, this optimized formulation delivers highly reliable quantitative analysis across various DNA templates.

2. KIT CONTENTS AND STORAGE

Contents	200 rxns	500 rxns
RealMOD™ Green T² 2X qPCR mix	1.0 ml x 2	1.0 ml x 5
Nuclease-free Water	1.0 ml x 2	1.0 ml x 5

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

3. PREPARATION OF qPCR REACTION MIXTURE

Components	Volume	Notes
RealMOD™ Green T² 2X qPCR mix	10.0 µl	Includes Hot-Start Taq
Primers (F / R, 10 µM)	0.4 µl / 0.4 µl	Default final concentration is 0.2 µM.
Template DNA / cDNA	Variable	Add optimal volume (≤ 2.0 µl for cDNA).
Nuclease-free Water	to 20.0 µl	Ultrapure liquid substrate.
Total Volume	20.0 µl	Mix gently by inverting, spin down.

NOTE: Do not vortex the master mix to avoid air bubbles, which will negatively affect the quantitative results. Mix by inverting and spin down briefly.

NOTE: Dilute the template prior to addition; undiluted cDNA template should be ≤ 1/10 of the total reaction volume.

4. INSTRUMENTATION PROGRAM SELECTION

Program	Temp.	Mode	Cycles
Initial Denaturation	95 °C	30 sec	1
Denaturation	95 °C	3-10 sec	40
Ann./Extension[1]	60 °C	10-30 sec*	
Melt Curve		Instrument Default	1

* Set the extension time to 10 seconds for amplicons ≤ 200 bp. For amplicons > 200 bp, 30 seconds is recommended.
[1] Configure your qPCR software to acquire real-time fluorescence signals (FAM channel) at this step.

5. TROUBLESHOOTING

Symptom	Possible Cause	Immediate Recommendation
No Amplification / Delayed Ct	• Signal acquisition step missed • Template degraded or low conc. • PCR inhibitors present • Custom Primer Tm Match	• Set signal acquisition at Annealing/Extension step. • Prepare fresh template or increase input. • Increase dilution factor to bypass inhibitors. • If custom primers fail at 60°C, optimize annealing down to 55°C.
Abnormal Plot Shape	• Bubbles remaining in the tube • Template concentration too high • Signal is too weak	• Spin down plates/tubes carefully before the run. • Reduce the baseline endpoint to (Ct value - 4). • Increase template concentration and retry.
Multiple Melt Peaks	• Inappropriate primer design • Primer concentration too high • gDNA contamination in cDNA	• Redesign primers (Length: 80–150 bp). • Decrease final primer concentration to 0.1–0.2 µM. • Increase annealing temperature by 1–2°C. • Prepare new cDNA to eliminate gDNA.
NTC Amplification	• Aerosol contamination • Primer-dimer formation	• Replace all reagents and use aerosol filter tips. • Prepare reaction mixtures strictly in a clean bench. • Check melting curve to distinguish dimers.
Poor Repeatability / Linearity	• Pipetting volume deviations • Low template concentration	• Use high-performance pipettes for exact volumes. • Increase template dilution factor and loading volume.

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