R3Charge Real-time One-Step Master Mix Protocol

For best results, keep all reagents on ice (or at 4˚C) at all times during set up.

Product Description

R3Charge 2× Green One-Step Mastermix (No ROX) is a high-performance reagent designed for single-tube reverse transcription and real-time PCR using intercalating dye detection chemistry. It enables both cDNA synthesis and quantitative amplification in one reaction, reducing handling steps, minimizing contamination risk, and improving reproducibility. This optimized 2× formulation contains all essential components for one-step RT-qPCR — reverse transcriptase, hot-start DNA polymerase, dNTPs, optimized buffer system, magnesium, stabilizers, and amplification enhancers — requiring only the addition of RNA template and primers. Engineered with a dual hot-start enzyme control system, the mix provides exceptional specificity, sensitivity, and consistent amplification across a wide range of RNA inputs. Its enhanced buffer chemistry and enzyme stability deliver robust performance for both low- and high-abundance targets, making it suitable for diverse gene expression studies and routine qPCR workflows. This formulation is ROX-free and optimized for real-time PCR instruments that detect fluorescence through intercalating dye signal. For systems requiring a passive reference dye, ROX may be supplemented according to instrument-specific guidelines (ROX not included).

Shipping & Storage Conditions

The product can be shipped using dry ice or blue ice. For optimal stability, store the master mix at –85 °C to –15 °C in a constant-temperature freezer. Avoid repeated freeze–thaw cycles by keeping it in working aliquots and protect it from direct sunlight. When stored as recommended, the product remains stable until its expiry date.

Real-time qPCR amplification and detection

1. Gently thaw RNA, primers/probes and R3Charge (2X) on ice. Vortex and spin down reagents.
2. Add RNA templates directly into plate.
3. Assemble qPCR reaction according to Table 1. Dispense appropriate volume of qPCR master mix per well.

Table 1 – Real-time qPCR setup, individual assays (96 well-plate)

Reagent	Volume
qPCR Master Mix (2x)	10 µl
qPCR Primers and Probes	(suggested final primer/probe concentration 400nM/200nM)
Nuclease-free water	to 20 µl
RNA template	X µl
Total volume	20 µl

4. Centrifuge the PCR plate briefly (30 s at 200 g).
5. Perform Real-time PCR amplification with the following cycling parameters.

Table 2 – qPCR fast thermal cycling protocol

Cycles	Temperature	Time	Notes
1x	50-55 °C	10-20 min	Reverse Transcription
1x	95 °C	2-5 min	Polymerase activation
40x	95 °C	5 s	Denaturation
	60 °C	30-60 s	Annealing/extension (acquire florescence reading at end of step) For amplicons >500bp, increase the annealing/extension time up to 1 min.

The master mix is compatible with real-time PCR instruments that do not require a passive reference signal for data normalization.

6. Data analysis

Ordering information

Real-time PCR Instrument	ROX requirement	Cat. No.	
		2X Green MM	2X Probe MM
Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® MIC qPCR Bio-Rad® iQ™5, CFX96, CFX384, Opticon Aperbio Pangaea	Not required	DS1272113 (600) DS1272114 (1,200) DS1272115 (2,400)	DS1272153 (600) DS1272154 (1,200) DS1272155 (2,400)
Applied Biosystems® 7500, QuantStudio™ 3, 5 and 7 ViiA7™ Agilent Mx™	Low ROX		DS1272133 (600) DS1272134 (1,200) DS1272135 (2,400)
Applied Biosystems® 7000, 7300 7700, 7900HT, StepOne™, StepOnePlus™	High ROX	DS1272123 (600) DS1272124 (1,200) DS1272125 (2,400)	DS1272143 (600) DS1272144 (1,200) DS1272145 (2,400)

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