Medix Biochemica

Product Manual
Cat. No: #6701

qRT-Probe Mix Separate ROX

Description

qRT-Probe Mix is a universal one-step probe mix for robust, sensitive, and fast RT-qPCR. The mix uses state-of-the-art technologies with an antibody-regulated hot-start Taq polymerase and reverse transcriptase for efficient cDNA synthesis and real-time PCR amplification in a single reaction chamber or tube. The optimized buffer chemistry and PCR enhancers, RNase inhibitor, and stabilizers enable rapid and sensitive RT-qPCR.

qRT-Probe Mix is compatible with several probes such as TaqMan® and Scorpions®. This allows rapid detection and quantification of a variety of RNA templates, such as mRNA, viral RNA, and total RNA. The kit includes an efficient thermostable reverse transcriptase with an RNase inhibitor (RTase Amp) to prevent degradation of RNA templates by RNases.

Kit Components

Component	S pack*	M pack*
qRT-Probe Mix 2x No ROX	1 mL	5 x 1 mL
20x RTase Amp	0.1 mL	0.5 mL
50 µM ROX Additive	0.2 mL	0.2 mL

*Other pack sizes and bulk orders are available upon request.

Storage and Shipment

Transport with an ice pack. The reagents should be stored at -20°C upon arrival. The reagents are stable until the expiration date if stored correctly. Do not store the mix once it is combined with the RTase.

Reaction Master Mix Set-Up

The recommended master mix set-up for a 20 µL reaction volume is shown in the table below.

For ROX additive use: If your qPCR instrument requires ROX correction, the 50 µM ROX Additive supplied is formulated to be added directly to the 1 mL tube of qRT-Probe Mix. Once the ROX is added, the reagent may be used straight away or stored at -20 °C for future use. Add 20 µL of ROX additive for HI-ROX or 2 µL of ROX additive for LOW-ROX to the 1 mL tube of qRT-Probe Mix. The final concentration after reaction set up will be 500 nM for HI-ROX and 50 nM for LOW-ROX instruments.

Reagent	Volume (µL)	Final concentration
qRT-Probe Mix 2x (ROX additive optional)	10	1x
∞Forward primer (10µM)	0.8 µL	400 nM
∞Reverse primer (10µM)	0.8 µL	400 nM
∞Probe (10µM)	0.4 µL	200 nM
20x RTase Amp	0.2	0.2x Alternatively titrate between 0.05 µL and 0.2 µL
RNA template	2-8	Variable
Nuclease-free Water	Up to 20 µL final volume	

∞Primers and probes should be specific to the target DNA/RNA of interest. The recommended Tm for primers is between 56°C and 60°C, and the Tm for probes should be between 65°C and 70°C.

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Instrument and Program Set-Up

Cycles	Steps	Temperature	Time
1	[Δ]Reverse Transcription	45–55°C	10 min
1	Polymerase activation	95°C	2 min
40	Denaturation	95°C	5 sec
	[ΔΔ]Annealing/ extension	60°C	30 sec

[Δ]The reverse transcription step should be performed at 45°C, except when the RNA template has a complex secondary structure. The reverse transcription time can also be increased to 20 minutes.

[ΔΔ]The annealing/extension step can be reduced to 20 seconds. Do not exceed 30 seconds, do not use temperatures below 60°C

Technical information and support

For technical enquiries or assay development support, please contact us via e-mail at: mdx@medixbiochemica.com.

Additional information and technical resources are available on our website at: info.medixbiochemica.com/resources.