

Concentration:

Standard RT-qULTRA™ OneStep

MasterMix: 2X Storage and Handling:

Store at -20°C upon arrival until provided expiration date. Minimize freeze/thaw cycles to avoid loss of performance.

Product Description:
2X Standard RT-qULTRA™ OneStep MasterMix:

This is a ready-to-use master mix for one-step, reverse-transcription quantitative PCR (RT-qPCR) detection of specific RNA sequences using dye or fluorescent probe-based detection. RT-qULTRA™ MasterMix contains RTScript™ reverse transcriptase and a chemically modified Taq DNA polymerase. The Taq polymerase remains completely inactive at room temperature and becomes activated after 2 minutes at 95°C. Empirical's RT-qULTRA™ MasterMix has been optimized for use with hydrolysis-based probes but is also suitable with other probe-based detection systems (i.e. molecular beacons) and dsDNA dye-based detection.

Protocol:

The following reaction setup and general cycling conditions are recommended but can vary depending on the template and primers being used. The following set up is for a 20µL reaction size.

Table 1: Example Set-up for 20µL Reaction

Component	Volume	Final Concentration
2X Standard RT-qULTRA™ OneStep MasterMix	10 µL	1X
Target Specific Primers and Probe	1 µL	1X
Sample containing RNA Template	X µL	100 ng to 1 pg
EvaGreen Dye, 20X in water (if required, purchase separately)	1 µL	1X
ROX Reference Dye, 25µM (see Table 2, if required, purchase	see Table 2	see Table 2
Nuclease Free Water to volume	X µL	Not Applicable
Total	20 µL	

Table 2: ROX Concentration Recommendation for Different Instruments

Type	Instrument		ROX final concentration
	Company	Instrument Name	
No ROX	Roche	LightCycler 480, LightCycler 2.0	None
	BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, Chromo4, MJ Opticon, Option2, MiniOpticon	
	Qiagen	Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000	
	Illumina	Eco RealTime PCR System	
	Eppendorf	Mastercycler realplex	
	Cepheid	SmartCyler	
Low ROX	ABI	7500, 7500 Fast	30nM
	Stratagene	MX4000P, MX3000P, MX3005P	
High ROX	ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus, Viia7	300nM

Table 3: Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
Reverse Transcription	1	1	50°C	15 minutes
Enzyme Activation	1	1	95°C	2 minutes
Amplification**	2	40	95°C	3 seconds
			60°C	30 seconds

**Temperature and Holding Time will be based off primer set used.