Product Information Sheet

Product Name: 2X qPCR Probe MasterMix* with 25µM ROX Reference Dye*

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Concentration:

qPCR Probe MasterMix: 2X
ROX Reference Dye: 25µM

Storage and Handling:

Upon arrival store at -20°C for provided expiration date, Room Temperature for up to 30 Days, 4°C for up to 60 days. Minimize Freeze thaw of MasterMix to avoid loss of performance.

Product Description:

qPCR Probe MasterMix:
Supplied as a 2x ready-to-use MasterMix for quantitative real time evaluation of DNA using fluorescent probe-based detection. Empirical's qPCR Probe MasterMix contains FlashTaq HotStart DNA Polymerase; a chemically modified Taq DNA polymerase that remains completely inactive at room temperature. The enzyme becomes activated after only 2 minutes at 95°C. Empirical's qPCR Probe MasterMix includes dNTPs, MgCl2, and optimized buffer for fast, efficient qPCR. Empirical's qPCR Probe MasterMix has been optimized for use with hydrolysis-based probes such as TaqMan but is also suitable with other probe-based detection systems.

25µM ROX Reference Dye:
Supplied separately for use with some real time machines.

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: Recommended Protocol for 20uL Reaction

PCR Component	Volume	Concentration
2X qPCR Probe MasterMix	10µL	1X
ROX reference dye, 25µM	X µl	Table 2
Primer, 10µM	X µl	0.1-0.5µM
DNA Template	X µl	0.01-100ng
Nuclease Free Water to volume	X µl	NA

Table 2: ROX concentration recommendation for different instruments

Type	Company	Instrument	Final Conc.
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

***This product is intended for Research Use Only. This product is manufactured under ISO13485:2016 Quality System Requirements and is available for use as a Raw Material for use in IVD applications. Please contact Empirical Bioscience for further details.**

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Recommended Thermal Cycling Conditions: The following general cycling conditions are recommended but can vary depending on the template and primers being used.

Table 3: Cycling Conditions

Cycling Step	Temperature	Holding Time	Cycles
Initial Denaturation	95°C	2 minutes	1
Denaturation	95°C	15 seconds	40
Annealing[#]	50-65°C	30-60 seconds	
Elongation	72°C	30 seconds	

[#]The annealing temperature depends on the melting temperature of the primer probe used.

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