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	<b>PIS-055 QP-MM-Reactions</b>	Version: 002 Effective Date: 07/06/20 Author: Beth Lowe CO#: 062520-1

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## Product Information Sheet

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

**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 30 Days, 4°C for up to 60 days.  
Minimize Freeze thaw of master mix to avoid loss of performance.

**Ordering Information:**

Item Number	Number of Tubes and Volume	Total number of reactions which can be obtained when using the following reaction sizes	
		20µL Reaction	10µL Reaction
QP-MM -500	2X qPCR Probe MasterMix :5 x 1mL, 25µM ROX Reference Dye: 1x150uL	500	1000
QP-MM -1000	2X qPCR Probe MasterMix: 10x1mL, 25µM ROX Reference Dye: 2x150uL	1000	2000
QP-MM -2500	2X qPCR Probe MasterMix: 25x1mL, 25µM ROX Reference Dye: 5x150uL	2500	5000

**Product Description:**

Empirical's qPCR Probe MasterMix is a 2x ready-to-use master mix 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, MgCl<sub>2</sub>, 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.

**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

\*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.  
For MSDS and Certificate of Analysis please visit [www.empiricalbioscience.com](http://www.empiricalbioscience.com)

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**Table 2: ROX concentration recommendation for different instrument**

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	SmartCycler	
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

**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 <sup>#</sup>	50-65°C	30-60 seconds	
Elongation	72°C	30sec	

<sup>#</sup>The annealing temperature depends on the melting temperature of the primer probe used.