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	PIS-078 ITP-MM-Reactions	Version: 002 Effective Date: 07/06/20 Author: Beth Lowe CO#: 062520-1

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Concentration: InhibiTaq PLUS HotStart qPCR MasterMix: 4X

Storage and Handling: Store at -20°C upon arrival

Ordering Information:

Item Number	Number of Tubes and Volumes	Total number of reactions which can be obtained when using the following reaction sizes	
		20µL Reaction	10µL Reaction
ITP-MM-200	4X InhibiTaq PLUS HotStart qPCR MasterMix: 1x1mL	200	400
ITP-MM-600	4X InhibiTaq PLUS HotStart qPCR MasterMix: 3x1mL	600	1200
ITP-MM-1000	4X InhibiTaq PLUS HotStart qPCR MasterMix: 5x1mL	1000	2000
ITP-MM-2000	4X InhibiTaq PLUS HotStart qPCR MasterMix: 10x1mL	2000	4000
ITP-MM-5000	4X InhibiTaq PLUS HotStart qPCR MasterMix: 25x1mL	5000	10000
ITP-MM-10000	4X InhibiTaq PLUS HotStart qPCR MasterMix: 50x1mL	10000	20000

Product Description:

4X InhibiTaq Plus HotStart qPCR MasterMix is an enhanced performance version of the 4X InhibiTaq HotStart qPCR MasterMix containing UDG Enzyme and dUTP to minimize the effects of carry over contamination. The Master Mix is a 4X ready-to-use MasterMix for quantitative real time evaluation of DNA using dye or fluorescent probe-based detection. InhibiTaq PLUS HotStart qPCR MasterMix contains 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 InhibiTaq Plus HotStart qPCR MasterMix has been optimized for use with hydrolysis-based probes but is also suitable with other probe-based detection systems and 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.

Reaction set-up for 20uL reaction volume Table 1:

Component	Volume	Final Concentration
4X InhibiTaq Plus HotStart qPCR MasterMix	5 µl	1X
Target Specific Primers and Probe	1 µl	1X
Sample	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 separately)	See Table 2	See Table 2
Nuclease Free Water to volume	X µl	N.A.
Total	20 µl	

*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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Table 2: ROX concentration recommendation for different instrument

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

Thermal cycling conditions:

Table 3: Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
UDG Incubation	1	1	25°C	2 minutes
Enzyme Activation	2	1	95°C	2 minutes
Amplification**	3	40	95°C	10 seconds
			60°C	60 seconds

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

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