Product Information Sheet

Product Name: 2X InhibiTaq™ Multiplex PLUS HotStart MasterMix *

Concentration:

InhibiTaq™ Multiplex PLUS HotStart 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 InhibiTaq™ Multiplex PLUS HotStart MasterMix:

This ready-to-use PCR master mix has been optimized for quantitative, real-time detection of specific DNA sequences in high-throughput settings where carryover contamination is a particular concern. InhibiTaq™ Multiplex HotStart PLUS MasterMix contains a chemically modified Taq DNA polymerase that remains completely inactive at room temperature and becomes activated after 2 minutes at 95°C. The PLUS version of this master mix also includes dUTP and a thermolabile uracil deglycosylase (UDG) enzyme that will degrade uracil-containing DNA amplicons to reduce interference from carryover contamination. Empirical's InhibiTaq™ Multiplex HotStart MasterMix has also been formulated for improved resistance to common biological and chemical inhibitors of PCR, making it suitable for direct qPCR amplification from unextracted sample types including biological swab material and saliva. The mix is optimized for use with hydrolysis-based probes but is also suitable with other probe-based detection systems (ie. 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 Reactions

Component	Volume	Final Concentration
2X InhibiTaq™ Multiplex PLUS HotStart MasterMix	10 µL	1X
Target Specific Primers	X µL	0.1-1.0µM
Target Specific Probe (if required)	X µL	0.1-1.0µM
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	see Table 2	see Table 2
Nuclease Free Water to volume	X µL	Not Applicable
Total	20 µL	

Table 2: ROX Concentration Recommandation 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

***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 3: Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
Enzyme Activation	1	1	95°C	2 minutes
Amplification**	2	40	95°C	10 seconds
			60°C	15 seconds

**Amplification temperature will depend on the predicted melting temperature of the primers used. Holding time should be determined based on amplicon length with an estimated 0.5 min/kb.

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