



Product Information Sheet

IBI KleenGreen qPCR 2X Master Mix

Concentration: 2X

Storage: Store at -20°C upon arrival. Minimize number of freeze/thaw cycles by storing in working aliquots. Avoid prolonged exposure to light.

Product Description: IBI KleenGreen qPCR 2X Master Mix is a ready-to-use HotStart qPCR master mix with EvaGreen dye, IBI Taq HotStart DNA Polymerase, 400µM dCTP, 400µM dGTP, 400µM dATP, 400µM dTTP, and 5mM MgCl₂. EvaGreen is a dsDNA-binding dye. It is cell membrane impermeable, non-cytotoxic, non-mutagenic, and safe for aquatic life. EvaGreen possesses similar absorption and emission spectra as SYBR[®] Green I. IBI Taq HotStart DNA Polymerase is an exceptional chemically modified HotStart Taq DNA Polymerase with an activation time of 2 minutes at 95°C. The Taq DNA Polymerase gene is isolated from *Thermus aquaticus* YT1 and expressed in *E.coli*. The recombinant Taq DNA Polymerase shows identical characteristics to native Taq from *Thermus aquaticus*. IBI KleenGreen qPCR 2X Master Mix is optimized to work with both fast (two step) and regular (three step) cycling conditions.

A 25µM ROX reference dye is supplied separately for use with some real time machines.

<u>Catalog #</u>	<u>Size</u>
IB43140	10 Reactions
IB43141	100 Reactions
IB43142	500 Reactions
IB43143	1000 Reactions

IBI#	Description	Size
IB43140	IBI KleenGreen qPCR 2X Master Mix (w/ROX)	10 RxNs
IB43141	IBI KleenGreen qPCR 2X Master Mix (w/ROX)	100 RxNs
IB43142	IBI KleenGreen qPCR 2X Master Mix (w/ROX)	500 RxNs
IB43143	IBI KleenGreen qPCR 2X Master Mix (w/ROX)	1000 RxNs

Protocol: The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

Table 1: Reaction Set-up For 20µl Reaction Volume

Component	Volume	Final Concentration
IBI KleenGreen qPCR 2X Master Mix	10µl	1X
ROX reference dye, 25µM	Xµl	Table 2
Primer, 10µM	Xµl	0.1 – 1.0µM
Template	Xµl	<250ng
Nuclease-free Water to:	20µl	N.A.



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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	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, Viiia7	300nM

Thermal Cycling Conditions:

Table 3: Two Step Fast Cycling

Cycling Step	Temperature	Holding Time	Cycles
Enzyme activation	95 ⁰ C	2 min	1
Denaturation	95 ⁰ C	5sec	40
Annealing & Extension	60 ⁰ C	30sec	

Table 4: Three Step Fast Cycling

Cycling Step	Temperature	Holding Time	Cycles
Enzyme activation	95 ⁰ C	2 min	1
Denaturation	95 ⁰ C	5sec	40
Annealing	55-60 ⁰ C	5sec	
Extension	72 ⁰ C	25sec	

Quality Control: IBI KleenGreen qPCR 2X Master Mix is tested and verified by Nuclease Assay, DNA Contamination Assay, and Activity (Functional) Assay. Complete results can be found on product C of A, available upon request.