Intact Genomics®

igSYBR™ Green qPCR 2X Master Mix

Catalog #	3355	3356
Package Size	100 reactions (20 µl rxn vol)	500 reactions (20 µl rxn vol)

Description

ig™ SYBR Green qPCR 2x master mix is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in qPCR. The ig™ SYBR Green qPCR 2x master mix with integrated chemically-modified hot start *Taq* DNA polymerase, SYBR Green I fluorescent dye, $MgCl_2$, dNTPs and stabilizers. ROX reference dye is not included in the 2x master mix*. This master mix is ideal for high-throughput real-time PCR screening and validation. The amplification step features a high quality hot start *Taq* DNA Polymerase which offers higher fidelity and better amplification.

Applications

- Gene expression data validation.
- Absolute quantification
- Mutation detection
- Pathogen detection
- viral detection
- Genetically modified organisms (GMO) characterization
- Genetic profiling

Benefits

- Enhanced efficiency, specificity, and sensitivity
- Compatible with all real-time PCR instruments
- Superior gene expression results under various cycling conditions
- Robust and active for cDNA synthesis at temperatures up to 55 °C.

Product Includes

1) ig™ SYBR Green qPCR 2x Master Mix

Storage Temperature

–20 °C

Note

*The use of ROX dye is necessary for all Applied Biosystems instruments and is optional for the Stratagene Mx3000P™, Mx3005P™, and Mx4000™ cyclers. Bio-Rad, Qiagen, Eppendorf, Illumina and Roche instruments do not require ROX dye.

Protocol

1. Place kit components, primers and RNA samples on ice.
2. Mix and then centrifuge briefly to collect contents at the bottom of the tube.
3. Prepare a master mix for each reaction and control plus 10% extra to allow for pipetting error according to the following table:
4. Mix the reaction mixture thoroughly.

PCR reaction set up:	
Diluted cDNA	1-5 µl
Forward primer (5 µM)	1.0 µl
Reverse primer (5 µM)	1.0 µl
ig™SYBR Green qPCR 2x master mix	10.0 µl
ROX (optional)	0.4 µl
H_2O up to	20.0 µl

5. Program the thermal cycler according to the manufacturer's instructions.
6. A typical PCR cycling program is outlined in the following table.

PCR Cycling Conditions:			
Steps	Temperature	Time	Cycles
Initial denaturation	95 ºC	15 min	1
Denaturation	95 ºC	5 sec	30-40
Annealing/Extension**	~60 ºC	30 sec	
Melting curve analysis	According to instrument guidelines		

7. Place the PCR tubes in the thermal cycler and start the cycling program.
8. Analyze the data according to manufacturer protocol.

** For 3 step cycling protocols, anneal at optimal annealing temperature for 30 sec followed by the minimum time required for data acquisition at 72 ºC according to instrument guidelines.