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Product Information

Version 6.1, Revision 2021-08-07

A4197 - iGreen[™] 2X qPCR MasterMix

iGreen™ 2X qPCR MasterMix provides a convenient, reliable and robust setup for performing quantitative real-time analysis of DNA samples. This ready-to-use qPCR MasterMix contains next generation iGreen[™] DNA Polymerase, providing for **rapid extension rates and robust performance**. With specialized reaction conditions, this polymerase provides increased processivity, yields, and sensitivity, while shortening reaction times by up to 70%, compared to wild-type Taq DNA polymerase.

iGreen[™] has 5'-3' polymerase and 5'-3' exonuclease activities, lacks 3'-5' exonuclease activity, and produces 3'-dA-tailed amplicons. qPCR products made with iGreen[™] can be used with TA cloning vectors.

Code	Product Component	Quantity
A4197	iGreen™ 2X qPCR MasterMix	500 rxn (4 x 1.25 ml)
	ROX Reference Dye	50 μl

Protocol

The recommended amount of ROX Reference Dye to be added into the MasterMix may vary depending on the qPCR machine type:

- No ROX equipment: Not needed.
- Low ROX equipment: $1 \mu l/1.25 ml$ or $22.5 \mu l/25 ml$ MasterMix.
- High ROX equipment: $11 \mu l/1.25 ml$ or $225 \mu l/25 ml$ MasterMix.

1. Mix individual components before use and assemble reaction on ice.

Component	Volume	
iGreen™ 2X qPCR MasterMix ¹	10 µl	
Forward Primer (10 μM)	0.5 μΙ	
Reverse Primer (10 μM)	0.5 μl	
Template DNA	Variable (100 ng genomic DNA)	
Nuclease-free H ₂ O up to 20 µl		

¹ The reaction buffer contains 1.5 mM Mg2+

2. Gently mix the reaction components and briefly centrifuge. Use thermocycling conditions below.

Step	Temperature	Duration (Standard)	Duration (Fast)	Cycles
Enzyme activation	95°C	3min	20sec	1
Denaturation	95°C	15sec	1sec	40
Anneal/Extension	60°C	1min	10sec	
Melting curve	Refer to specific guidelines for instrument used			

General Notes

- Specialized buffer for higher yields, sensitivity, and specificity compared to wild-type Taq polymerase.
- Ideally start the qPCR as soon as the reaction mixture is prepared. If not possible, keep the reaction mixture on ice until starting the qPCR.
- Use the fast thermocycling condition with miRNA cDNA templates or any other appropriate applications.

This material is for laboratory research purpose and/or in vitro use only and is not to be used in humans or animals.