

## BrightGreen 2X qPCR MasterMix

Store at -20°C

Cat. No.	Product Name	Quantity
MasterMix-R-XL	BrightGreen 2X qPCR MasterMix - ROX	2000X20 µl rxns (20 ml)
MasterMix-LR-XL	BrightGreen 2X qPCR MasterMix - Low ROX	2000X20 µl rxns (20 ml)
MasterMix-iC-XL	BrightGreen 2X qPCR MasterMix - iCycler	2000X20 µl rxns (20 ml)
MasterMix-S-XL	BrightGreen 2X qPCR MasterMix - No Dye	2000X20 µl rxns (20 ml)

### Product Description

A 2X concentrated mix of Taq polymerase, dNTPs, MgCl<sub>2</sub>, fluorescent dye (detection), reference dye and proprietary buffer components, the BrightGreen 2X qPCR MasterMix provides a convenient, reliable and robust set-up for performing quantitative real-time analysis of DNA samples. Designed specifically for the aforementioned niche of application, the components of BrightGreen 2X qPCR MasterMix promise top notch performance with respect to sensitivity, signal-to-noise ratio and elimination of primer dimers. Furthermore, **crb's** proprietary chemical modification of the DNA polymerase included in this MasterMix allows for Hot Start PCR, conferring a significant reduction in non-specific PCR amplification that is otherwise of a common occurrence with regular Taq polymerases.

In light of the fact that the qPCR instruments can vary from user to user, **crb** offers the BrightGreen 2X qPCR MasterMix in a range of formulations, each of which has been carefully optimized to confer the best performance according to the make and model of a qPCR machine. Please use the following table as a guide for selecting the qPCR MasterMix that will be most compatible with your choice of a particular instrument/model.

Cat. No.	Product Name	qPCR Instrument(s)
MasterMix-R-XL	BrightGreen 2X qPCR MasterMix - ROX	2000X20 µl rxns (20 ml)
MasterMix-LR-XL	BrightGreen 2X qPCR MasterMix - Low ROX	2000X20 µl rxns (20 ml)
MasterMix-iC-XL	BrightGreen 2X qPCR MasterMix - iCycler	2000X20 µl rxns (20 ml)
MasterMix-S-XL	BrightGreen 2X qPCR MasterMix	2000X20 µl rxns (20 ml)

### Product Application(s)

The BrightGreen 2X qPCR MasterMix is ideally suited for:

- Gene expression analysis
- Microarray validation
- Viral load determination

### Storage Conditions

Upon arrival, the BrightGreen 2X qPCR MasterMix should be stored at -20°C and protected from light. After each experiment, the leftover mix (completely thawed and thoroughly homogenized) can be stored at 4°C if it is to be used within the next 3 months. Avoid repeated freeze-thaw cycles to retain maximum performance. The BrightGreen 2X qPCR MasterMix is stable for 1 year from the date of shipping when stored and handled properly.

### Protocol

1. Thaw the BrightGreen 2X qPCR MasterMix, template DNA, primers and nuclease-free water on ice. Mix each solution thoroughly.

Note: Please ensure no salt crystals are present in the BrightGreen 2X qPCR MasterMix before use. If salt crystals are observed, mix until crystals are completely dissolved and absent.

2. Set up the following reaction mixture (10 µl or 20 µl reaction volume):

Components	10 µl Reaction	20 µl Reaction	Final Concentration
BrightGreen 2X qPCR MasterMix	5 µl	10 µl	1X
Forward Primer (10 µM)	0.3 µl	0.6 µl	300 nM
Reverse Primer (10 µM)	0.3 µl	0.6 µl	300 nM
Template DNA	Variable	Variable	≤500 ng/reaction
Nuclease-free H <sub>2</sub> O	to 10 µl	to 20 µl	

3. Perform qPCR reactions using the following cycling program:

Step	Temperature	Duration (Standard)	Duration (Fast)	Cycle(s)
Enzyme activation	95°C	10 mins	20 secs	1
Denaturation	95°C	15 secs	3 secs	40
Annealing/Extension	60°C	60 secs	30 secs	
Melting curve	Refer to specific guidelines for instrument used			

### Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- Note that the BrightGreen 2X qPCR MasterMix components are light sensitive and therefore, avoid prolonged exposure to direct light.
- Ideally, start the PCR as soon as the reaction mixture is prepared. If not, then make sure that the reaction mixture is kept chilled till starting up the PCR.