Owi Green FAST qPCR Premix

Follow these 3 steps for a successful test

References: 258493 - 258494 - 258495

For RESEARCH USE ONLY

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1. Choose the most appropriate ROX Reference Dye for your QPCR machine.

Two different ROX Reference Dye are supplied in the kit. Please refer to the table below for Rox Dye usage details:

ROX types	qPCR Instruments
No ROX	Bio-Rad iCycler™ series, Roche LightCycler® series Qiagen/Corbett series and others
ROX Reference Dye I	Applied Biosystems™ 7000/7300/7700/7900HT, Applied Biosystems™ StepOne™/StepOnePlus™ Eppendorf and others
ROX Reference Dye II	Applied Biosystems™ 7500/7500Fast, ABI ViiA™ 7, Applied Biosystems™ QuantStudio™, Stratagene series, Corbett Rotor-Gene® 3000P™ and others

2. Prepare the qPCR reaction mix: use 0,2 µM of each primer for the first assay.

Reagent	Volume	Final Concentration
2X Owi Green FAST qPCR Premix	10 µL	1X
Forward Primer (10 µM)	0.4 µL	**0.2 µM**
Reverse Primer (10 µM)	0.4 µL	**0.2 µM**
Template DNA (< 50 ng)	≤ 2 µL	< 50 ng / rxn
50X ROX I or 50X ROX II (optional)	0.4 µL	1X
Nuclease free ddH_2O	up to 20 µL	up to 20 µL

Dominique DUTSCHER SAS - 67170 BERNOLSHEIM - FRANCE
Tel. : +33 3 88 59 33 90 – www.dutscher.com
Customer service - Order : info@dutscher.com
Technical support : demandes_techniques@dutscher.com

3. Set the qPCR program :

Stage 1	Pre-denaturation	1 cycle	95 °C	**3 min.**
Stage 2	Cycles	40-45 cycles	95 °C	5 sec.
			60 °C	**30-34 sec.***
Stage 3	Melting Curve	1 cycle	Default	

** Make sure that signal acquisition is performed during the extension step. The extension time should be adjusted according to the minimum signal acquisition time, which depends upon the qPCR instrument you use.* ***E.g., set to 30 sec. for StepOne Plus™, 31 sec. for ABI 7300 and 34 sec. for ABI 7500.***

An additional note for small reaction volume (ex. 10 µl): 2X Owi Green FAST qPCR Premix contains glycerol. Mix smoothly before use, avoiding bubbles, and centrifuge to collect the complete Premix volume at the bottom of the tube.

§ "Difficult targets" often require protocol optimization. We recommend that you include well known standard genes for your first test run. Please contact our Technical Service if you need additional advice for difficult targets.

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