Owi Green FAST qPCR Premix

User Manual

References: 258493 - 258494 - 258495

For RESEARCH USE ONLY

DOMINIQUE DUTSCHER SAS

SIZE: 1 ml - 100 reactions (20 µL) / 5 ml - 500 reactions (20 µL) / 5 x 5 ml - 2500 reactions (20 µL)

STORAGE: -20℃ in the dark

SHELF-LIFE: one year from the date of reception when stored properly

PRODUCT DESCRIPTION:

The **Owi Green FAST qPCR Premix** is designed for intercalator-based real-time PCR using Owi Green Dye. This product provides real-time data on DNA amplification during PCR by performing quantitative fluorescence signal detection on SYBR™/FAM channels. This premix includes ROX reference dye in two separate tubes and thus suits all currently used qPCR instruments (including high ROX, low ROX and no ROX modes required instruments). It includes also an antibody-based hot-start Taq DNA Polymerase for amplification, which greatly improves the product specificity while ensuring the amplification efficiency.

This product is supplied at 2X concentration and contains all the components required for qPCR except primers and templates, providing great convenience for experimental manipulation.

LIST OF COMPONENTS:

Component	1 mL	5 mL
2X **Owi Green FAST qPCR Premix***	1 mL	5 mL
50X ROX Reference Dye I	50 µL	220 µL
50X ROX Reference Dye II	50 µL	220 µL

*contains HotStart Taq DNA polymerase, Mg^{2+}, dNTPs, Owi **Green**, reaction buffer

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COMPATIBLE INSTRUMENTS:

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

Note: Different ROX Reference Dyes can be selected based on the type of qPCR instrument. Please refer to the above table for ROX dye usage.

PREPARE:

1. Corresponding Eppendorf tubes, PCR tubes, pipettes and tips, ice box or ice.
2. qPCR primers and corresponding templates.
3. Fluorescence quantitative PCR dedicated tubes or plates and sealed optical films.

PRECAUTIONS:

1. When using 2X **Owi Green FAST qPCR Premix**, please fully thaw it before use and keep it away from light. Extending exposure time to light will result in lower fluorescent signals.
2. The 2X **Owi Green FAST qPCR Premix** contains glycerol. Mix gently to avoid air bubbles before use. After mixing, collect all the liquid to the bottom of the tube by centrifugation. After use, immediately return the premix to -20°C freezer.
3. This premix contains polymerase. Please prepare qPCR reaction mix on ice. For short time reuse, stored it temporarily at 4 °C. Please avoid repeated freezing and thawing.
4. This product includes two different ROX reference dyes in separate tubes. Select the right ROX reference dye based on the type of qPCR instrument (please refer to “Compatible Instruments” table).
5. Please use proper non-contaminating pipette tips. The tips including filters are recommended to minimize cross-contamination when preparing qPCR reaction mixes.
6. To ensure successful reaction, high quality DNA templates are recommended.

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PROTOCOL:

Before experiment:

1. Please ensure the specificity of the primers. A recommended starting primer concentration of 0.2 µM will provide good performance for most of the targets. Additional primer titrations from 0.1 µM to 1.0 µM will help to optimize reaction efficiency without compromising specificity.
2. The amplicon length is recommended to be in the range of 70 bp to 200 bp.
3. It is recommended to establish a standard curve using serial dilutions of the template DNA.
4. It is recommended to add 1 pg - 50 ng of template DNA (genomic or plasmid) to 20 µL reaction mixture and always set a no template control (NTC). For two-step RT-qPCR reactions, the cDNA template volume should not exceed 10% of the final qPCR reaction volume. Alternatively use serial dilution to identify the optimal template volume.
5. To ensure experimental reproducibility, it is recommended to set triplicates for each sample and control.

For qPCR experiment:

1. Prepare the following reaction mix on ice:

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

1) Thaw the 2X **Owi Green FAST qPCR Premix** at room temperature. When it is completely thawed, mix the premix carefully to avoid bubbles, centrifuge to collect all the liquid to the bottom of the tube. Keep it on ice.

2) Calculate the total volume of qPCR reaction mix required for the experiment. Typically, add an extra 10% to the theoretical total volume to compensate pipetting loss. Accurately aliquot qPCR mix to individual clean tube; avoid liquid contamination
3) Add respectively the corresponding primers and template to each reaction tube or well. After all the reagents (qPCR premix, primers, template, ROX when needed, water) have been added, mix well and centrifuge immediately.

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4) Transfer the above reaction mix to a dedicated qPCR plate and carefully seal it with an optical sealing film (ensure as much as possible that no bubbles are formed during the transfer and no contact between liquid and film).

5) Centrifuge the qPCR plate at 2500 rpm and collect all the reaction solution to the bottom of the wells. The qPCR plate are ready for thermocycling.

2. Set the qPCR program

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

* Make sure that signal acquisition is performed in the extension step. The extension time should be adjusted according to the minimum signal acquisition time by the real time qPCR instrument you use. Set to 30 sec. for StepOnePlus™, 31 sec. for ABI 7300, and 34 sec. for ABI 7500.

DATA ANALYSIS:

1. A standard curve is generated based on the C_t value and the sample input. The standard curve correlation coefficient (R^2) should be >0.98. The standard curve slope should be in the range of -3 and -3.5. The PCR amplification efficiency (E) is generally between 90 and 120%.
2. The standard deviation of the C_t value among the triplicates is typically <0.2.
3. Ideally, there is no multiple peaks or primer dimer peak in the melting curve. If necessary, you can also check the qPCR products by running agarose gel electrophoresis. T_m value of the melting curve is generally from 80°C to 95 °C.
4. Confirmation of valid C_t values: the C_t value of the valid amplification should be at least 5 cycles less than the C_t value of the no-template control curve, and the melting curve should have no multiple peaks.

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