WizPure™ qPCR Master (SYBR) Lo-ROX

RUO For Research Use Only

REF W1711RL

DESCRIPTION

WizPure™ qPCR Master (SYBR) Lo-ROX kit is an optimized ready-to- use 2X master mix solution for real-time quantitative PCR assays. It comprises all the components necessary to perform qPCR: antibody hot-start Taq DNA Polymerase, ultrapure dNTPs, MgCl2, SYBR Green I, enhancer, stabilizer and ROX reference dye ensures that the WizPure™ qPCR Master (SYBR) Lo-ROX kit delivers fast, highly-specific and ultra-sensitive real-time PCR.

KIT CONTENTS

Contents	W1711RL	W1711RL-5
qPCR Master (SYBR) Lo-ROX	1 ml	5 X 1 ml

APPLICATIONS

- Real-time PCR
- Detection and quantification of DNA and cDNA targets
- Gene expression profiling
- Array validation

STORAGE CONDITIONS

All kit components should be stored at -20°C upon receipt.
Excessive freeze/thawing is not recommended.

NOTE

Do not contaminate the WizPure™ qPCR Master (SYBR) Lo-ROX kit with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

INSTRUMENT COMPATIBILITY

WizPure™ qPCR Master (SYBR) Lo-ROX kit has been optimized for use in SYBR green-based real-time PCR on the real-time PCR instruments listed in the followings;

- ABI (Thermofisher) : 7500, 7500 FAST, ViiA7, QuantStudio
- Roche : LightCycler 480, LightCycler 2.0
- Stratagene (Agilent) : Mx3000P, Mx3005P and Mx4000
- Qiagen : Rotor-Gene Q

QUALITY CONTROL ANALYSIS:

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPure™ qPCR Master (SYBR) Lo-ROX kit is tested against predetermined specifications to ensure consistent product quality.

PROTOCOL

Prior to the experiment, it is prudent to carefully optimize experiment conditions and to include controls at every stage. See pre-protocol considerations for details.
This standard protocol applies to a single reaction where only template, primers, probe and water need to be added to the qPCR Master (SYBR) Lo-ROX mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
3. The following table shows recommended component volumes:

Component	20 µl reaction	Final Conc.
qPCR Master (SYBR) Lo-ROX	10.0 µl	1X
10µM Forward Primer	0.2-2.0 µl	0.1-1.0 µM
10µM Reverse Primer	0.2-2.0 µl	0.1-1.0 µM
Template DNA	Variable	≤ 500 ng/reaction
Water, RNase-Free	up to 20 µl	NA

NOTE: In general, use greater than 0.5 µM primers for sensitivity and less than 0.5 µM for specificity.
NOTE: Recommended amount of template per PCR reaction:

- < 50 ng plasmid or
- < 500-1000ng genomic DNA or
- 2µl of a 100µl single plaque eluate or
- one single bacterial colony

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
   (Optional) Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
5. Transfer tubes into a Real-time PCR instrument and run as following table.

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10 - 30 sec.	30 - 40
Anneal	55 - 68	10 - 60 sec.	
Melting curve analysis	65 - 95	2-5 sec./step	1

NOTE: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.
NOTE: Shorter annealing step time (<10sec.) can be used for amplicon <100bp.

ORDERING INFORMATION

Product	Cat No.	Package
WizPure™ qPCR Master (SYBR) Lo-ROX	W1711RL	100 rxn
	W1711RL-5	500 rxn

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