USER GUIDE: EBL SYBR® qPCR Master Mix(2X)

An BioReagent for molecular biology, Storage at -20 - 4 °C

About the Kits:

Cat.	Description	Qty.	qPCR Instruments
AQP0100R AQP2500R	SYBR® qPCR Master Mix (2X) w/ ROX	1 ml 25 x 1 ml	ABI,7000,7300,7700, 7900 stepOne Plus, StepOne™ Eppendorf Realplex 4 ABI7500, Stratagene Mx3000, Mx3005, Mx4000
AQP0100 AQP2500	SYBR® qPCR Master Mix (2X) w/o ROX	1 ml 25 x 1 ml	BioRad CFX96 Roche LightCycler 480 MJ Research Opticon and Opticon 2 MJ Research Chromo 4 Corbett Rotor-gene 600,3000 Eppendorf Realplex 2 Product Application

Components:

SYBR® qPCR Master Mix (2X) is a 2X mix of dNTPs, Hotstart Taq polymerase, $MgCl_2$, fluorescent detection dye, reference dye (optional), and proprietary buffer components.

Recommended Protocol:

Thaw SYBR® qPCR Master Mix (2X), template DNA, RNase-free water and primer on ice. Mix each solution well. Prior to the experiment, It is prudent to carefully optimize experiment Conditions and to Include controls at every stage. See preprotocol considerations for details. This standard protocol applies to a single reaction where only template, primers and water need to be added to the SYBR® qPCR Master Mix (2X). 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.
3. Prepare a reaction Master mix using the following:

Components	Volume/Reaction	Final Concentration
SYBR® qPCR Master Mix (2X)	10-25 µl	1X
Primer A	Variable	100-500nM
Primer B	Variable	100-500nM
Sterile water	Variable	
Template	Variable	≦ 500 ng
Total Volume	20-50 µl	

Two-step fast cycling protocol:

This cycling protocol should be applicable to most amplifications where the primer Tm's are designed to be 60 °C. Melt curves may be performed by following instructions provided for your instrument.

Step	Temperature	Duration	Cycles
Enzyme activation	95 °C	10 min	1
Denature	95 °C	5 sec	40-45
Anneal / extension	60 °C	30 sec	
Melting curve	According to the instrument guidelines		

Three-step fast cycling protocol:

This cycling protocol can be used if you would like to have the extension step to be performed at a higher temperature than the annealing step. For example, if you have relatively long primers that tend to anneal non-specifically, carrying out the extension step at a higher temperature can reduce nonspecific amplification. Melt curves may be performed by following instructions provided for your instrument.

Step	Temperature	Duration	Cycles
Enzyme activation	95 °C	10 min	1
Denature	95 °C	5 sec	40-45
Anneal	60 °C	5 sec	
Extension	72 °C	25 sec	
Melting curve	According to the instrument guidelines		

Recommendations for Optimal Results :

- Aliquot reagents to avoid contamination and to avoid repeated freeze-thaw cycles.
- SYBR® qPCR Master Mix (2X) components are light sensitive; avoid exposure to light.
- Start PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to PCR reactions.

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.