SB qPCR Master Mix (2X), Rox

QP1313-01 2x1.25 mL. QP1313-02 10x1.25 mL

Storage and Stability
Biomiga SB-Green qPCR Master Mix, rox, is stable for 1 year when stored at -20°C, protected from light. For convenience it may be stored at 4°C for up to 6 months. Repeated freezing and thawing of the mix is not recommended. However, no significant performance loss was detected after 10 times freeze-thaw cycles.

Description
Biomiga SB-Green qPCR Master Mix, rox, a ready-to-use reaction cocktail that contains all components except primers and template for real-time quantitative PCR on systems that require rox input. The proprietary buffer and stabilizers have been optimized to deliver maximum PCR efficiency, sensitivity and robust fluorescent signal. The proprietary 2X qPCR Master Mix, Rox contains DnaUs Hot Start *Taq* DNA polymerase, dNTPs, Mg^{2+}, enhancer, stabilizer, and an optimal concentration of ROX for instruments that require ROX for a reference signal. Please consult our website and Instrument Compatibility section below for the correct product for your real-time PCR system.

Hot Start *Taq* DNA polymerase is an antibody-inactivated hot-start enzyme designed to block polymerase activity at ambient temperature. Once the PCR step reaches denaturation temperature (95ºC), *Taq* DNA polymerase activity is restored and the resulting PCR exhibits higher sensitivity, specificity and yield.

The system enables highly sensitive detection from few copies of DNA template, with a broad dynamic range that supports accurate quantification of target sequences. Sufficient reagents are provided for 50, 200 or 500 amplification reactions of 50 µl each.

Features
- High yield, high sensitivity
- Superior reliability and robustness
- Convenient ready-to-use ROX containing qPCR Master Mix
- Ideal for fluorogenic probe chemistries including TaqMan probes.

Product Qualification
Biomiga SB-Green qPCR Master Mix, rox, is functionally tested in qPCR. Kinetic analysis demonstrate liner resolution over six orders of dynamic range (R^2>0.995) and RT-PCR efficiency >90%.

Kit components

	100 Rxns	500 Rxns
• **SB-Green qPCR Master Mix, rox(2X)** (Contains Hot Start Taq, dNTPs, 6 mM Mg^{2+}, SYBR Green 1 Dye, Enhancer and Stabilizers)	2X1.25ml	10X1.25ml

Recommended qPCR Reaction Protocol
The following protocol is suggested as a starting point. For multiple reactions, prepare a master mix of common components by adding the appropriate volume to each tube or plate well on ice, and then add the unique reaction components (*e.g.,* DNA template).

1. Thaw all components at room temperature. Mix gently, then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing.

2. Assemble reactions on ice or at room temperature.

Components	50 µl Rxn	20 µl Rxn	Final Concentration
LeGene qPCR Master Mix, Rox (2X)	25 µl	10 µl	1X
Forward Primer (10 µM)	1.0 µl	0.4 µl	200 nM (Variable:100-900 nM)
Reverse Primer (10 µM)	1.0 µl	0.4 µl	200 nM (Variable:100-900 nM)
Fluorogenic probe (10 µM)	0.5 µl	0.2 µl	100 nM (Variable:100-250 nM)
Template	x µl	x µl	Variable (genomic DNA, cDNA, plasmid DNA)
Volume with nuclease-free water	to 50 µl	to 20 µl	

Note: *Reaction volume can be scaled from 5 - 50 µl depending on the reaction plate (i.e. 384-well vs. 96-well) and qPCR system. Scale all component volumes proportionally. Addition of template as 5 to 10µL volumes will improve assay precision. A maximum of 10% of the qPCR reaction volume may be undiluted cDNA.*

3. Seal the tubes or wells with optically transparent caps or film. Mix gently, then centrifuge to remove any air bubbles and collect contents in the vessel bottom.

4. Program your real-time instrument as shown below. Optimal temperatures and incubation times may vary.

	Standard Cycling	Fast Cycling
Taq activation	95°C, 2 min	95°C, 2 min
PCR cycling (30-45 cycles)	95°C, 10-15s 60°C, 30-60s (Data collection)	95°C, 3-5s 60°C, 20-30s (Data collection)

Melting curve analysis (Refer to instrument documentation).

Optimal cycling conditions will vary for different primer sets.

5. Place reactions in your real-time instrument programmed as described above. Collect data and analyze results.

Recommendations and Guidelines for qPCR

Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the Bio-Rad CFX96 Touch, CFX384 Touch, CFX Connect, the Corbett Research Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Illumina Eco qPCR, etc.

Reagent	Catalog Number	Compatible Real-Time PCR System
Biomiga SB-Green qPCR Master Mix	QP1311	Bio-Rad CFX96, CFX384, CFX Connect; the Corbett Research Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Illumina Eco qPCR; Roche LightCycler™ 480; Eppendorf Mastercycler® ep realplex
Biomiga SB-Green qPCR Master Mix, Rox	QP1312	ABI® 7500, 7500 Fast, ViiA 7 Real-Time PCR Systems, the Stratagene Mx3000P®, Mx3005P™, and Mx4000®; the Agilent AriaMx
Biomiga SB-Green qPCR Master Mix, Rox	QP1313	ABI® 7000, 7300, 7700, 7900, 7900HT, StepOne™, StepOnePlus™ Real-Time PCR Systems

Template

A maximum of 10% of the qPCR reaction may be undiluted cDNA. Suggested input of cDNA is cDNA corresponding to 1pg to 100ng of total RNA. The suggested input of genomic DNA is 100pg to 100ng and 10-10^7 copies of plasmid DNA.

Magnesium Concentration

The 2X Reaction Mix includes magnesium at a final concentration of 3 mM. This works well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the following table to determine the amount of $MgCl_2$ to add to achieve the specified concentration:

For a Final $MgCl_2$ Concentration of	Add this Volume of 50 mM $MgCl_2$ (per 50 µl Rxn)
4.0 mM	1 µl
5.0 mM	2 µl
6.0 mM	3 µl 96

Melting Curve Analysis

Melting curve analysis should always be performed during qPCR to identify the presence of primer dimers and analyze the specificity of the reaction.

Primers

The design of highly specific primers is very important for successful qPCR with SYBR Green. For best qPCR efficiency, primers designed for amplicon sizes of 70-150bp. A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 100 and 500 nM.

Troubleshooting Guide

Problem	Possible Cause	Solution
No amplification curve appears on the qPCR graph	There is no PCR product	Run the reaction on a gel to determine whether the PCR worked. Then proceed to the troubleshooting steps below.
No PCR product appears on the qPCR graph or on a gel.	The protocol was not followed correctly.	Verify that all steps have been followed, and that correct reagents, volumes, and cycling conditions were used.
	Annealing temperature and time may be suboptimal.	Perform an annealing temperature gradient. Increase annealing step in 3s intervals up to 30s.
	Amplicon too long	Amplicons should ideally be 70-150bp long and should not exceed 500bp.
	Template contains inhibitors, nucleases, proteases, or has been degraded.	Purify, or if needed, re-purify your template.
	Primer design is not optimal.	Verify your primer selection. The use of validated pre-designed primers, or design of primers using dedicated software programs or primer databases is recommended.
PCR product appears on the gel, but not on the qPCR graph.	qPCR instrument settings are incorrect.	Confirm that you are using the correct instrument settings, (dye selection, reference dye, filters, etc).
	Problems with your qPCR instrument.	Refer to your instrument manual for troubleshooting tips.
Signal is present in no template controls, and or multiple peaks are present in the melting curve graph.	Template or reagents are contaminated by DNA or cDNA.	To identify the contaminants use the melting curve analysis on your machine, or run the PCR products on a 4% agarose gel after the reaction. When preparing your reactions, take standard precautions to avoid contamination. The use of aerosol-resistant barrier tips, and assembly in a DNA-free environment are recommended.
	Primer dimers or other primer artifacts are present	To identify primer dimers use melting curve analysis. The use of validated pre-designed primer sets or designed primers from dedicated software programs or primer databases are recommended. Check the purity of your primers by gel electrophoresis. Primer contamination or truncated or degraded primers can lead to artifacts.
PCR efficiency is above 110%.	Template contains inhibitors, nucleases, proteases, or has been degraded.	Purify, or if needed, re-purify your template. Inhibitors in the template may result in changes in PCR efficiency between dilutions.
	Nonspecific products may be amplified.	To identify the contaminants use the melting curve analysis on your machine, or run the PCR products on a 4% agarose gel after the reaction. Verify your primer selection. The use of validated pre-designed primers, or design of primers using dedicated software programs or primer databases is recommended
PCR efficiency is below 90%	PCR conditions are not optimal.	Verify the reagents you are using have not been freeze-thawed multiple times and have not remained at room temperature for too long. Verify that the amount of primers you are using is correct.
	Template contains inhibitors.	Purify, or if needed, re-purify your template.

Poor linearity of Ct values across dilution series (R value ≤ 0.998)	Too much nucleic acid in "high copy number" assays	Use less than 500ng of template in your reaction.
	Too little nucleic acid present in "low copy number" assays	Increase the amount of template or increase PCR reaction efficiency by optimizing thermal protocol/ re-designing primers.
	Reaction components not thoroughly mixed.	Repeat assay ensuring all serial dilutions are vortexed for 15s and reaction components are thoroughly mixed.
	Poor template quality	Check the quality of the template preparation using spectrophotometry, microfluidics or PAGE.