OriGene Technologies, Inc.
9620 Medical Center Drive, Suite 200
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Tele: 888.267.4436
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SensiFAST SYBR Master Mix - Lo ROX Kit

Store at –20°C

Shipping: On Blue Ice

Lot Number: See Vial

Catalog Numbers

QP100016: 100 Reactions (1.0 mL)

QP100017: 500 Reactions (5.0 mL)

QP100018: 1000 Reactions (10 mL)

Storage and Stability:
The SensiFAST SYBR Master Mix - Lo ROX kit is shipped on Blue Ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended. Since SYBR® Green I is light-sensitive, it is important to avoid prolonged exposure to light. When stored under optimum conditions, the reagents are stable for a minimum of 6 months from date of purchase.

Safety Precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Description

The SensiFAST SYBR Master Mix - Lo ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR instruments. A combination of the latest advances in buffer chemistry and enhancers, together with an antibody-mediated hot-start DNA polymerase system, ensures that the SensiFAST SYBR Master Mix - Lo ROX Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.

For ease-of-use and added convenience, SensiFAST SYBR Master Mix - Lo ROX kit is provided as a 2x mastermix containing all the components necessary for real- time PCR, including the SYBR® Green I dye, dNTPs, stabilisers and enhancers. As a ready-to-use premix, only primers and template need to be added.

Kit components

Reagent	100 x 20µl Reactions	500 x 20µl Reactions	1000 x 20µl Reactions
Cat. Number	QP100016	QP100017	QP100018
SensiFAST SYBR Master Mix - Lo-ROX (2x	1 x 1.0 ml (1.0 ml)	5 x 1.0 ml (5.0 ml)	10 x 1.0 ml (10.0 ml)

Instrument compatibility

SensiFAST SYBR Master Mix - Lo ROX Kit has been optimized for use in SYBR Green-based real-time PCR on the real-time PCR instruments listed in the following compatibility table, each of these instruments having the capacity to analyze the real-time PCR data with the passive reference signal either on or off. The kit is also compatible with several instruments that do not require the use of ROX, such as the Qiagen (Corbett) Rotor-Gene™ 6000, the Bio-Rad CFX96 or the Roche LightCycler® 480.

Manufacturer	Model
ABI (Invitrogen)	7500, 7500 FAST, ViiA7
Stratagene (Agilent)	Mx4000™, Mx3000P™, Mx3005P™

General considerations

To help prevent any carry-over DNA contamination we recommend that separate areas be maintained for PCR set-up, PCR amplification and any post-PCR gel analysis. It is essential that any amplified PCR product should not be opened in the PCR set-up area.

Primers: the sequence and concentration of primer and the amplicon length can be critical for specific amplification, yield and overall efficiency of any real-time PCR. We strongly recommend you use OriGene's validated primer pairs when designing and running your PCR reactions.

Template: it is important that the DNA template is suitable for use in PCR in terms of purity and concentration. Also, the template needs to be devoid of any contaminating PCR inhibitors (e.g. EDTA). We recommend OriGene's 1st Strand cDNA Synthesis Kits (NP100041 and NP100042). The recommended amount of template for PCR is dependent upon the type of DNA used. The following should be considered when using genomic DNA and cDNA templates:

- **cDNA:** the optimal amount of cDNA to use in a single PCR is dependent upon the copy number of the target gene. We suggest using 100ng cDNA per reaction, however it may be necessary to vary this amount. To perform a two-step RT-PCR, we recommend using OriGene 1st Strand cDNA Synthesis Kits (NP100041 and NP100042) for reverse transcription of the purified RNA. For high yield and purity of RNA, use OriGene Total RNA Purification Kits (NP100026 and NP100027)

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of real-time PCR conditions this is optimal for both the reverse transcriptase and the hot-start DNA polymerase.

PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no template control (NTC), replacing the template with PCR-grade water. When performing a two-step RT-PCR, set-up a no RT control as the NTC for the PCR.

Optional ROX: The SensiFAST SYBR Lo-ROX Kit is premixed with ROX (5-carboxy-X-rhodamine, succinymidyl ester), so that ROX fluorescence can be optionally detected on certain real-time PCR instruments. If your real-time PCR instrument has the capability of using ROX and you wish to use this option, then this option must be selected by the user in the software.

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Procedure

Reaction mix composition: Prepare a PCR master mix. The volumes given below are based on a standard 20µl final reaction mix and can be scaled accordingly.

Reagent	Volume	
2x SensiFAST SYBR Lo ROX Mix	10µl	1x
10µM forward primer	0.8µl	400nM
10µM reverse primer	0.8µl	400nM
Template	up to 8.4µl	
H_2O	As required	
	20µl Final volume	

Sensitivity testing and C_t values: When comparing SensiFAST with a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of speed. In some instances increasing final $MgCl_2$ concentration to 6mM will reduce Cts for difficult amplicons.

Suggested real-time PCR conditions: The following real-time PCR conditions are suitable for the SensiFAST SYBR Master Mix - Lo ROX Kit with amplicons up to 200bp. However, the cycling conditions can be varied to suit different machine-specific protocols. It is not recommended to use annealing temperatures below 60°C or combined annealing/extension times longer than 30 seconds.

SensiFAST SYBR Master Mix - Lo ROX Kit is compatible with either three-step or two-step cycling:

3-step cycling

Cycles	Temp.	Time	Notes
1	*95°C	*2min	Polymerase activation
40	95°C 60-65°C 72°C	5s 10s **5-20s	Denaturation Annealing Extension (acquire at end of step)

***2min for cDNA, 3min for genomic DNA**
****Not recommended to extend beyond 20 seconds**

2-step cycling

Cycles	Temp.	Time	Notes
1	*95°C	*2min	Polymerase activation
40	95°C 60-65°C	5s **15-30s	Denaturation Annealing/extension (acquire at end of step)

***2min for cDNA, 3min for genomic DNA**
****Not recommended to anneal/extend beyond 30 seconds**

Optional analysis: After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	For cDNA templates, make sure SensiFAST SYBR Master Mix - Lo ROX Kit is activated for 2min at 95°C before cycling. For more complex templates such as genomic DNA, increase inactivation time up to 3minutes.
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer design software or validated primers. Test primers on a control template
	Incorrect concentration of primers	Use primer concentration between 100nM and 1µM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
	Primers degraded	Use newly synthesized primers
	Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR-grade H_2O
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number
No amplification trace AND Product on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling

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Troubleshooting Guide (Continued)

Problem	Possible Cause	Recommendation
Non-specific amplification product AND Primer-dimers	Suboptimal primer design	Redesign primers using appropriate software or use validated primers
	Primer concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primer concentration too low	Titrate primers in the concentration range of 100nM - 1µM
	Primer annealing/extension temperature(s) too low	Due to the high ionic strength of SensiFAST SYBR - Lo ROX Kit, it is not recommended to use annealing/extension temperatures below 60°C. Annealing/ extension temperature can be increased in steps of 2°C in the event of non-specific products
	Template concentration too low	Increase template concentration
	Template concentration too high	Reduce template concentration until non-specific products disappear
	Extension time too long	Reduce extension time to determine whether non-specific products are reduced
Late amplification trace	Activation time too short	Ensure the reaction is activated for between 1min and 3min at 95°C before cycling
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C
	Extension time too short	Increasing the extension time may be necessary for amplification products over 200bp; double extension time to determine whether the cycle threshold (Ct) is affected
	Template concentration too low	Increase concentration if possible
	Template with high secondary structure	Increase reverse transcription reaction time up to 30min Increase reverse transcription reaction temperature up to 45°C
	Template is degraded	Re-isolate template from sample material or use freshly prepared template dilution
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
	Primer concentration too low	Increase concentration of primer in 100nM increments
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer concentration too low	Increase concentration of primer in 100nM increments
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
PCR efficiency above 110%	Template is degraded or contains PCR inhibitors	Re-isolate template from sample material or use freshly prepared template dilution or purify template and resuspend it in H_2O
	Non specific amplification and/or primer dimers	Use melt analysis and 4% agarose gel electrophoresis to confirm presence of non-specific amplification products. See above for preventing/removing non-specific products

TRADEMARK AND LICENSING INFORMATION

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Purchase of this product includes limited right to use the supplied amount of SYBR® Green I Stain patented by Molecular Probes, Inc.

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