OriGene Technologies, Inc.
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SensiMix SYBR Master Mix - Low ROX Kit

Shipping: On Blue Ice

Lot Number: See vial

Catalog Numbers

QP100004: 100 Reactions (1.25 mL)

QP100005: 400 Reactions (5 mL)

QP100006: 1600 Reactions (20 mL)

Store at –20°C

Storage and Stability:
The SensiMix SYBR Master Mix –Low 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 SensiMix SYBR Master Mix – Low ROX Kit is a high-performance reagent designed for superior sensitivity and specificity on various real-time instruments, in which a ROX passive reference signal is required. The SensiMix SYBR Master Mix – Low ROX Kit uses the hot-start properties of our proprietary DNA polymerase, StarTaq, for high specificity and sensitivity of the PCR. StarTaq is inactivated and possesses no polymerase activity during the reaction set-up, preventing non-specific amplification including primer-dimer formation.

For ease-of-use and added convenience, qSTAR SYBR Master Mix – Low ROX is provided as a 2x master mix 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 25µl Reactions	400 x 25µl Reactions	1600 x 25µl Reactions
Cat. Number	QP100004	QP100005	QP100006
SensiMix SYBR Master Mix – Low ROX (2x)	1 x 1.25ml (1.25ml)	4 x 1.25ml (5ml)	16 x 1.25ml (20ml)

Kit compatibility

SensiMix SYBR Master Mix – Low ROX (2x) Kit contains premixed ROX fluorescent dye for compatibility with real-time instruments that acquire a ROX passive reference signal for normalization of the data. The SensiMix SYBR Master Mix – Low ROX Kit is optimized for use on the real-time instruments listed in the following compatibility table.

Manufacturer	Model
ABI	7500
Stratagene	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 as well as 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 reaction.

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 First 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 First 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)

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.

Procedure

Reaction mix composition: Prepare a PCR master mix. The volumes given below are based on a standard 25µl final reaction mix and can be scaled accordingly.

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Reagent	Volume	Final concentration
2x SensiMix SYBR Master Mix – Low Rox	12.5µl	1x
10µM Primer Mix	1.0µl	400nM
H_20	Up to 22.5µl	-
Template	2.5µl	
Final volume	**25µl**	

Suggested thermal cycling conditions

The PCR conditions described are suitable for SensiMix SYBR Master Mix – Low ROX Kit for the majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit customer or machine-specific protocols. The critical step of the PCR is the 10 minute initial activation at 95°C. The detection channel on the real-time instrument should be set to (SYBR) Green or FAM.

Standard 3-step cycling

Cycles	Temperature	Time	Notes
1	*95°C	*10min	Polymerase activation
40	95°C 55-60°C 72°C	15s 15s 15s	Temp. depends on the Tm of primers Acquire at end of step

2-step cycling

Cycles	Temperature	Time	Notes
1	*95°C	*10min	Polymerase activation
40	95°C 60°C	15s 30s	Acquire at end of step

***Non-variable parameter**

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	Make sure SensiMix is activated for 10min at 95°C before cycling
	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, reduce annealing temperature
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 temperature too low	Increase PCR annealing temperature in increments of 2°C until primer dimer/non-specific amplification products disappear
	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 10min at 95°C before cycling
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C
	Extension time too short	Double extension time to determine whether the cycle threshold (C_T) 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