SensiFAST™ SYBR No-ROX Kit

Shipping: On Dry/Blue Ice

Exp. Date: See vial

Batch No.: See vial

Concentration: see vial

Catalog Numbers

BIO-98002: 200 x 20µl reactions: 2 x 1ml

BIO-98005: 500 x 20µl reactions: 5 x 1ml

BIO-98020: 2000 x 20µl reactions: 20 x 1ml

BIOLINE

A Meridian Life Science® Company

Store at –20°C

Storage and Stability:
The SensiFAST SYBR No-ROX Kit is shipped on Dry/Blue Ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended. When stored under optimum conditions, the reagents are stable for a minimum of 12 months from date of purchase.

Quality Control:
Bioline operates under ISO 9001 Management System. The SensiFAST SYBR No-ROX Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

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

Notes:
For research use only.

Description

The SensiFAST SYBR No-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time 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 No-ROX Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.

For ease-of-use and added convenience, SensiFAST SYBR No-ROX 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. The kit consists of a ready-to-use premix, only primers and template need to be added.

Kit components

Reagent	200 x 20µl Reactions	500 x 20µl Reactions	2000 x 20µl Reactions
SensiFAST SYBR No-ROX mix (2x)	2 x 1ml	5 x 1ml	20 x 1ml

Instrument compatibility

The SensiFAST SYBR No-ROX Kit is compatible with real-time instruments that do not need a passive reference signal for normalization of the data. The SensiFAST SYBR No-ROX Kit has been optimized for use on the real-time instruments listed in the following compatibility table.

Manufacturer	Model
Bio-Rad	Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384
Cepheid	SmartCycler™
Qiagen	Rotor-Gene™ 3000 & 6000
Eppendorf	Mastercycler® ep realplex
Roche	LightCycler® 480
Techne	Quantica®
Illumina®	Eco™
Takara	Thermal Cycler Dice® (TP800)

General considerations

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

Primers: The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the primers, as well as by the amplicon length. We strongly recommend taking the following points into consideration when designing and running your real-time PCR:

- use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMP™ (http://dnasoftware.com/). Primers should have a melting temperature (Tm) of approximately 60°C.
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- optimal amplicon length should be 80-200bp, and should not exceed 400bp
- final primer concentration of 400nM is suitable for most SYBR-Green based reactions, however to determine the optimal concentration we recommend titrating in the range 0.1-1µM
- use an equimolar primer concentration
- when amplifying from cDNA,use of intron spanning primers to is preferable, to avoid amplification from genomic DNA

Template: it is important that the DNA template is suitable for use in PCR in terms of purity and concentration. In addition, the template needs to be devoid of any contaminating PCR inhibitors (e.g. EDTA). The recommended amount of template for PCR is dependent upon the type of DNA used. The following points should be considered when using genomic DNA and cDNA templates:

- **Genomic DNA:** use up to 1µg of complex (e.g. eukaryotic) genomic DNA in a single PCR. We recommend using the Bioline ISOLATE Genomic DNA Mini Kit (BIO-53021) for high yield and purity from both prokaryotic and eukaryotic sources
- **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 the Tetro cDNA Synthesis Kit (BIO-65042) for reverse transcription of the purified RNA. For high yield and purity of RNA, use the Bioline ISOLATE RNA Mini Kit (BIO-54042)

$MgCl_2$: 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 well as an NTC for the PCR.

Procedure

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

Reagent	Volume	Final concentration
2x SensiFAST SYBR No-ROX Mix	10µl	1x
10µM Forward Primer	0.8µl	400nM
10µM Reverse Primer	0.8µl	400nM
Template	As required	
H_2O	up to 20µl	

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 C_t 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 C_ts for difficult amplicons.

Suggested real-time PCR conditions: The following real-time PCR conditions are suitable for the SensiFAST SYBR No-ROX Kit with the amplicons of 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 No-ROX Kit is compatible with either 3-step or 2-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 No-ROX is activated for 2min at 95°C before cycling. For more complex templates such as genomic DNA, increase inactivation time up to 3 minutes.
	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 water
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number

Troubleshooting Guide (Continued)

Problem	Possible Cause	Recommendation
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
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 No-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
Variability between replicates	Error in reaction set-up	Prepare large volume mastermix, vortex thoroughly and aliquot into reaction plate
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument
Late amplification trace	Activation time too short	Ensure the reaction is activated for between 1min and 3min at 95°C before cycling
	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 (C_t) is affected
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C
	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
	$MgCl_2$ concentration is insufficient	Increase final $MgCl_2$ concentration to 6mM
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 water
	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

Associated Products

Product	Description	Pack Size	Cat No.
ISOLATE Genomic DNA Mini kit	Rapid isolation of DNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52031 BIO-52032 BIO-52033
ISOLATE Plant DNA Mini kit	Rapid isolation of DNA from a variety of plant samples	10 Preps 50 Preps 250 Preps	BIO-52034 BIO-52035 BIO-52036
ISOLATE RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52039 BIO-52040 BIO-52041
ISOLATE Plant RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of plant samples	10 Preps 50 Preps 250 Preps	BIO-52042 BIO-52043 BIO-52044
TRIsure™	Quick isolation of high-quality RNA from a variety of sources for subsequent use in cDNA synthesis	100ml 200ml	BIO-38032 BIO-38033
Tetro cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length cDNA from RNA	30 Reactions 100 Reactions	BIO-65042 BIO-65043
Agarose	Molecular biology grade agarose	100g 500g	BIO-41026 BIO-41025
PCR Water	Ultra-pure (18.2MΩ) molecular biology grade water	10 x 10ml	BIO-37080
DEPC-treated Water	Deionized, high-quality molecular grade water treated with DEPC. Ideal for use in all RNA work	10 x 10ml 1 Liter	BIO-38030 BIO-38031

Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

Email: tech@bioline.com

Trademark and Licensing Information

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