SensiFAST™ Probe Lo-ROX Kit

Shipping: On Dry/Blue Ice

Exp. Date: See vial

Batch No.: See vial

Concentration: see vial

Catalog Numbers

BIO-84002: 200 x 20µl reactions: 2 x 1ml

BIO-84005: 500 x 20µl reactions: 5 x 1ml

BIO-84020: 2000 x 20µl reactions: 20 x 1ml

Store at –20°C

Storage and Stability:
The SensiFAST Probe Lo-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 6 months from date of purchase.

Quality Control:
Bioline operates under ISO 9001 Management System. The SensiFAST Probe Lo-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.

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DATA SHEET

Description

The SensiFAST™ Probe Lo-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time instruments. The kit has been formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. A combination of the latest advances in buffer chemistry and PCR enhancers, together with a hot-start DNA polymerase, ensures that the SensiFAST Probe Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.

SensiFAST Probe is provided as a 2x mastermix containing all the components necessary for real-time PCR, including dNTPs, stabilizers and enhancers.

Kit components

Reagent	200 x 20µl Reactions	500 x 20µl Reactions	2000 x 20µl Reactions
SensiFAST Probe Lo-ROX mix (2x)	2 x 1ml	5 x 1ml	20 x 1ml

Instrument compatibility

SensiFAST Probe Lo-ROX Kit has been optimized for use with all probe chemistries, including TaqMan, FRET, Scorpions and molecular beacon probes on real-time 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 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 and probe: These guidelines refer to the design and set-up of TaqMan probe-based PCR. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the probes and 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; the Tm of the probe should be approximately 10°C higher than that of the primers
- optimal amplicon length should be 80-200bp, and should not exceed 300bp
- final primer concentration of 400nM is suitable for most Probe-based reactions, however to determine the optimal concentration we recommend titrating in the range 0.2-1µM
- use an equimolar primer concentration
- a final probe concentration of 100nM is suitable for most applications; we recommend that the final probe concentration is at least 2-fold lower than the primer concentration
  Note: In multiplex qPCR probe concentrations over 100nM can result in cross-channel fluorescence

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 Bioline cDNA Synthesis Kit (BIO-65026) 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 SensiFAST Probe mix contains an optimized concentration of $MgCl_2$, it is not necessary to supplement the mix further.

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.

Optional ROX: The SensiFAST Probe Lo-ROX Kit is premixed with ROX (5-carboxy-X-rhodamine, succinymidyl ester), so that where necessary, ROX fluorescence can be optionally detected on certain real-time instruments. If your real-time 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 (*see notice to purchaser No. 4 in Trademark and Licensing Information*).

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 Probe Lo-ROX Mix	10µl	1x
10µM Forward Primer	0.8µl	400nM
10µM Reverse Primer	0.8µl	400nM
10µM Probe	0.2µl	100nM
H_2O	up to 16µl	
Template	4µl	
	20µl Final volume	

Suggested thermal cycling conditions

The real-time PCR conditions, in the table below, are suitable for the SensiFAST Probe Lo-ROX Kit with the amplicons of up to 200bp. These cycling parameters have been optimized on a number of platforms, however they can be varied to suit different machine-specific protocols.

Cycles	Temperature	Time	Notes
1	95°C	*2-5min	Polymerase activation
40	95°C	10s	Denaturation
	60°C	**20-50s	Annealing/extension (acquire at end of step)

***2min for cDNA, up to 5min for genomic DNA**
****Up to 50s may be necessary for multiplexing with more than 2 probes**

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 Probe Lo-ROX is activated for 2min at 95°C before cycling. For more complex templates such as genomic DNA, increase activation time up to 5 minutes
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer/probe design software or validated primers. Test primers on a control template
	Incorrect concentration of primers/probe	Use primer concentration between 300nM and 1µM and probe concentration of 100nM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
	Primers/probe degraded	Use newly synthesized primers and probe
	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/probe design	Use primer/probe design software or validated assays. Test primer/probe on a control template
	Primer/probe concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primer/probe concentration too low	Use primer concentration between 300nM and 1μM and probe concentration of 100nM
	Primer annealing/extension temperature(s) too low	Due to the high ionic strength of SensiFAST Probe 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
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 2min and 5min 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 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/probe concentration too low	Use primer concentration between 300nM and 1μM and probe concentration of 100nM
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer/probe concentration too low	Use primer concentration between 300nM and 1μM and probe concentration of 100nM
	Suboptimal design of primers/ probe	Use primer/probe design software or validated assays. Test primer/probe on a control template

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
cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length cDNA from RNA	30 Reactions 100 Reactions	BIO-65025 BIO-65026
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.