SensiMix™ II Probe Lo-ROX Kit

Shipping: On Dry/Blue Ice

Batch No.: See vial

Concentration: See vial

Catalog Numbers

BIO-88005: 500 x 50µl reactions: 10 x 1.25ml

BIO-88020: 2000 x 50µl reactions: 40 x 1.25ml

BIOLINE

A Meridian Life Science® Company

Store at –20°C

Storage and Stability:
SensiMix II 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.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Quality Control:
SensiMix II 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:
Please refer to the material safety data sheet for further information.

Notes:
For research use only.

Description

SensiMix™ II Probe Lo-ROX Kit is a high-performance reagent designed for superior sensitivity and specificity on all real-time instruments. The kit has been formulated for use with probe-detection technology, including TaqMan®, Scorpion®, Assay On Demand®, allelic discrimination and molecular beacon probes. SensiMix II Probe Lo-ROX Kit employs a hot-start DNA polymerase, for high PCR specificity and sensitivity. Since the polymerase possesses no activity during reaction set-up, the kit greatly reduces non-specific amplification including primer-dimer formation. After pre-heating, the polymerase becomes fully activated and in conjunction with a specially optimized buffer chemistry, generates reliable and highly reproducible data on all real-time PCR instruments.

For ease-of-use and added convenience, SensiMix II Probe Lo-ROX Kit is provided as a 2x mastermix containing all the components necessary for real-time PCR, including dNTPs and stabilizers.

Kit components

Reagent	500 x 50µl reactions	2000 x 50µl reactions
SensiMix™ II Probe Lo-ROX (2x)	10 x 1.25ml (12.5ml)	40 x 1.25ml (50ml)
50mM $MgCl_2$	1 x 1ml	4 x 1ml

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 tube containing amplified PCR product should not be opened in the PCR set-up area.

Primers and probe: These guidelines refer to the use of TaqMan probes. Please refer to the relevant literature when using other probe types. The sequence and concentration of the probe and primers, as well as amplicon length, can be critical for specific amplification, yield and overall efficiency of any real-time PCR. We strongly recommend taking the following into consideration when designing and running your PCR reaction:

- use primer-design software, such as Primer3 or visual OMP™ (http://frodo.wi.mit.edu/primer3/ and DNA Software, Inc http://dnasoftware.com/ respectively). Primers should have a melting temperature (Tm) of approximately 58-60°C. The Tm of the probe should be approximately 10°C higher than that of the primers
- optimal amplicon length should be 80-150bp and should not exceed 400bp
- a final primer concentration of 400nM is suitable for most probe reactions, however to determine the optimal concentration we recommend titrating in the range of 0.3 - 1.0 µM
- use equimolar primer concentrations
- 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 PCR probe concentrations over 100nM can result in cross-channel fluorescence
- when amplifying from cDNA use intron-spanning primers 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. Also, 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 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 II Genomic DNA Mini Kit (BIO-52067) 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 SensiFAST cDNA Synthesis Kit (BIO-65053) for reverse transcription of the purified RNA. For high yield and purity of RNA, use the Bioline ISOLATE II RNA Mini Kit (BIO-52072)

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of qPCR conditions this is optimal for both the reverse transcriptase and the hot-start DNA polymerase. If necessary, we suggest titrating the $MgCl_2$ to a maximum of 5mM.

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.

Kit compatibility

The SensiMix II Probe Lo-ROX Kit can be used on instruments that do not require the use of ROX (5-carboxy-X-rhodamine, single isomer), such as the BioRad® Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384, iQ5™, Cepheid® SmartCycler™, Qiagen (Corbett) Rotor-Gene™ 3000, 6000 & Q, Analytik Jena qTower2, Eppendorf Mastercycler ep Realplex, ep Realplex 2S, Roche LightCycler® 480, LightCycler® Nano, Techne Quantica®, PrimeQ, Illumina Eco™, Takara Thermal Cycler Dice® TP800.

Optionally, the SensiMix II Probe Lo-ROX Kit can also be used in real-time PCR on some instruments (ABI 7500, 7500 Fast, ViiA7™, Agilent Mx3000P™, Mx30005P™, Mx4000™) that offer the user the choice of analyzing the real-time PCR data with the passive reference signal either on or off. 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.

Procedure

The following are instructions for the use of TaqMan probes in real-time PCR. Please refer to the relevant protocols when using other probe types.

Reaction mix composition: Prepare a PCR mastermix. The volumes given below are based on a standard 50µl final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
2x SensiMix™ II Probe Lo-ROX	25µl	1x
10µM Forward Primer	2µl	400nM
10µM Reverse Primer	2µl	400nM
10µM Probe	0.5µl	100nM
H_2O (BIO-27080)	up to 45µl	
Template	5µl	
	50µl Final volume	

If using the ABI Pre-developed TaqMan Assay Reagents (TaqMan PDARs) for allelic discrimination use genomic DNA in the range 10-100ng per 50µl final reaction mix.

Suggested thermal cycling conditions: The following PCR conditions are suitable for SensiMix II Probe Lo-ROX Kit with a majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit different probe-based reactions 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 acquire at the appropriate wavelength(s).

- **Standard cycling**

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

***Non-variable parameter**

- **Fast cycling**

Cycles	Temperature	Time	Notes
1	*95°C	*10min	Polymerase activation
40	95°C 60°C	10s 20s	Acquire at end of step

***Non-variable parameter**

It is important, when using the ABI TaqMan PDARs for allelic discrimination, to increase the extension temperature in the standard cycling profile from 60°C to 65°C.

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	Make sure SensiMix II 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 primers/probe design	Use primers/probe design software or validated assays. Test assay on a control template
	Incorrect concentration of primers/probe	Use primer concentration between 300nM and 1µM and probe concentration at 100mM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
	Primers/probe degraded	Use newly synthesized primers/probe
	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

Troubleshooting Guide (Continued)

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Non-specific amplification product AND Primer-dimers	Suboptimal primers/probe design	Redesign primers/probe using appropriate software or use validated assays
	Primers/probe concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primers/probe concentration too low	Increase concentration of primer and probe in 100nM increments
	Primers/probe 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 that 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 is degraded	Re-isolate template from sample material or use freshly prepared template dilution
	Suboptimal design of primers/probe	Redesign primers/probe using appropriate software or use validated primers
	Primers/probe concentration too low	Increase concentration of primer and probe in 100nM increments

Technical Support

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

Email: tech@bioline.com

Associated Products

Product	Description	Pack Size	Cat No.
ISOLATE II Genomic DNA Kit	Rapid isolation of high-quality genomic DNA from many different starting material	10 Preps 50 Preps 250 Preps	BIO-52065 BIO-52066 BIO-52067
ISOLATE II Plant DNA Kit	Rapid isolation of high-quality genomic DNA from a wide variety of plant species	10 Preps 50 Preps 250 Preps	BIO-52068 BIO-52069 BIO-52070
ISOLATE II RNA Mini Kit	Isolation of high-yield and extremely pure total RNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52071 BIO-52072 BIO-52073
ISOLATE II RNA Plant Kit	Isolation of high-yield and extremely pure total RNA from a wide variety of plant species	10 Preps 50 Preps	BIO-52076 BIO-52077
TRIsure™	Quick isolation of high-quality RNA from a variety of sources for subsequent use in cDNA synthesis	100ml 200ml	BIO-38032 BIO-38033
SensiFAST™ cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length and low abundance cDNA from RNA	50 Reactions 250 Reactions	BIO-65053 BIO-65054
Agarose	Molecular biology grade agarose	100g 500g	BIO-41026 BIO-41025

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