

SensiMix™ II Probe Kit

Shipping: On Dry/Blue Ice Catalog Numbers

Exp. Date: See vial BIO-91002: 250 x 50µl reactions: 5 x 1.25ml

Batch No.: See vial BIO-91005: 500 x 50µl reactions: 10 x 1.25ml

Concentration: see vial BIO-91020: 2000 x 50µl reactions: 40 x 1.25ml

Store at -20°C

Storage and Stability:

The SensiMix™ II Probe 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:

The SensiMix II Probe 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.



DATA SHEET

Description

The SensiMix™ II Probe 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. The SensiMix II Probe Kit employs a hot-start DNA polymerase, for high PCR specificity and sensitivity. Since SensiMix possesses no polymerase activity during reaction set-up, the kit greatly reduces non-specific amplification including primer-dimer formation. After pre-heating, SensiMix 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 is provided as a 2x mastermix containing all the components necessary for real-time PCR, including dNTPs and stabilizers. In addition a separate tube of ROX is provided for optional use.

Kit components

Reagent	250 x 50µl reactions	500 x 50µl reactions	2000 x 50µl reactions
SensiMix™ II Probe (2x)	5 x 1.25ml (6.25ml)	10 x 1.25ml (12.5ml)	40 x 1.25ml (50ml)
25µM ROX dye	250µl	500µl	4 x 500µl
50mM MgCl ₂	1 x 1ml	1 x 1ml	4 x 1ml

Kit compatibility

The SensiMix II Probe Kit has been optimized for use with all probe chemistries, including TaqMan®, Scorpion®, Assay On Demand®, allelic discrimination and molecular beacon probes.

The SensiMix II Probe Kit can be used on all real-time PCR instruments.

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 (T_m) of approximately 58-60°C. The T_m 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 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 (Tetro) 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)

MgCl₂: The MgCl₂ 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 MgCl₂ 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.

Optional ROX: Reaction-independent ROX fluorescence can be measured on the real-time instruments listed below to normalize the reporter-dye signal during PCR. SensiMix II Probe Kit is supplied with a separate tube of ROX (5-carboxy-X-rhodamine, succinimidyl ester) at 25µM. Use the following table to determine the appropriate volume of 25µM ROX, per 50µl reaction, to use with the particular real-time instrument:

Manufacturer	Model	ROX volume 50µl reaction	Final ROX concentration
ABI	7000, 7300, 7700, 7900, 7900HT, StepOne™, StepOne™ Plus	1.0µl	500nM
	7500, 7500 FAST, ViiA7	0.1µl	50nM
Stratagene	Mx4000™, Mx3000P™, Mx3005P™	0.1µl	50nM

Table 1.

Alternatively add 50µl of the 25µM ROX dye to each of the 1.25ml SensiMix II Probe(2x) for a 500nM final concentration of ROX, or 5µl of 25µM ROX dye to each of the 1.25ml SensiMix II Probe(2x) for a 50nM final concentration of ROX.

(see notice to purchaser No. 5 in Trademark and Licensing Information).

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.

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No amplification trace	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
AND No product on agarose gel	Incorrect concentration of primers/probe	Use primer concentration between 300nM and 1µM and probe concentration at 100nM
	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 ₂ O
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number, reduce annealing temperature

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

(*see ROX passive reference selection above)

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 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	10s	Acquire at end of step
	60°C	60s	

*Non-variable parameter

• Fast cycling

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

*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 (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 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

TRADEMARK AND LICENSING INFORMATION

1) Trademarks: SensiMix™ (Bioline Reagents Ltd.), SYBR® (Molecular Probes), ROX™, PRISM® (Applied Biosystems), iCycler™ Mx3005™, Opticon™, Chromo4™, MiniOpticon™, (Bio-Rad), LightCycler™ (Roche), TaqMan®, Assay On Demand®, StepOne™, ViiA7™ (ABI), SmartCycler™ (CEPheid), RotorGene™ (Corbett), RealPlex™ (Eppendorf), Quantica™ (Techne), MX4000 (Stratagene), Scorpion® (DxS).

2) Notice to Purchaser: Limited License. Use of this product is covered by one or more of the following US patents: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152 (claims 1 to 23 only), 5,773,258 (claims 1 and 6 only). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as the patented 5' Nuclease Process claims in US Patents Nos. 5,210,015 and 5,487,972) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

3) Notice to Purchaser: PCR probes can be purchased from a variety of vendors including Applied Biosystems (Life Tech), Roche Molecular Systems, Inc., F. Hoffman La-Roche Ltd., Integrated DNA Technologies, Biosearch Technologies, Nanogen Inc. and others. The use of certain probes including TaqMan-MGB, FAM-TAMRA, FAM-BHQ, VIC-MGB in connection with the Polymerase Chain Reaction ("PCR") process may require a license from one or more of these vendors. Please contact individual vendors to determine the requirement to obtain licenses. The purchase of this kit, as supplied by Bioline does not, either expressly or by implication, provide a license to use any proprietary technology supplied by these vendors.

4) SensiMix products are manufactured by Bioline Reagents Ltd.

5) Notice to Purchaser: No rights are conveyed with respect to US patent 5,928,907