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	100 mL 200 mL	BIO-38032 BIO-38033
SensiFAST™ cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length cDNA from RNA	50 Reactions 250 Reactions	BIO-65053 BIO-65054
Agarose	Molecular biology grade agarose	100 g 500 g	BIO-41026 BIO-41025

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.

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SensiMix™ II Probe Kit

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.

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:
The SensiMix II Probe Kit and it's 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 or further manufacturing use only.

Trademarks:
SensiMix (Bioline Reagents Ltd.), ROX (Roche), TaqMan (ABI)

Shipping: On Dry/Blue Ice
Batch No.: See vial
Concentration: See vial

Catalog Numbers
BIO-83005: 500 x 50 µL reactions: 10 x 1.25 mL

Store at –20°C

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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 master mix containing all the components necessary for real-time PCR (qPCR), including dNTPs and stabilizers. In addition a separate tube of ROX is provided for optional use.

Kit components

Reagent	500 x 50 µL reactions
SensiMix™ II Probe (2x)	10 x 1.25 mL (12.5 mL)
25 µM ROX dye	500 µL

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 qPCR. 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 80-150 bp and should not exceed 400 bp
- a final primer concentration of 400 nM 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 100 nM 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 100 nM 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 1 µg of complex (e.g. eukaryotic) genomic DNA in a single PCR. We recommend using the 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 100 ng cDNA per reaction, however it may be necessary to vary this amount. To perform a two-step RT-PCR, we recommend using the SensiFAST cDNA Synthesis Kit (BIO-65053) or reverse transcription of the purified RNA. For high yield and purity of RNA, use the ISOLATE II RNA Mini Kit (BIO-52072)

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3 mM. 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 5 mM.

Probe Compatibility:
The kit has been optimized for use with TaqMan®, Scorpian®, Assay on Demand®, allelic discrimination, and molecular beacon probes.

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-qPCR, 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 25 µM. Use the following table to determine the appropriate volume of 25 µM ROX, per 50 µL 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	1.0 µL	500 nM
	7500, 7500 FAST, ViiA7	0.1 µL	50 nM
Stratagene	Mx4000™, Mx3000P™, Mx3005P™	0.1 µL	50 nM

Table 1.

Alternatively add 50 µL of the 25 µM ROX dye to each of the 1.25 mL SensiMix II Probe (2x) for a 500nM final concentration of ROX, or 5 µL of 25 µM ROX dye to each of the 1.25 mL SensiMix II Probe (2x) for a 50 nM 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 qPCR. Please refer to the relevant protocols when using other probe types.

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

Reagent	Volume	Final concentration
2x SensiMix™ II Probe	25 µL	1x
10 µM Forward Primer	2 µL	400 nM
10 µM Reverse Primer	2 µL	400 nM
10 µM Probe	0.5 µL	100 nM
25 µM ROX* (see Table 1.)	-	-
H_2O	up to 45 µL	
Template	5 µL	
	50 µL 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 10-100 ng per 50 µL 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	*10 min	Polymerase activation
40	95 °C 60 °C	10 s 60 s	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	10 s 20 s	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 300 nM and 1 µM and probe concentration at 100 mM
	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
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 100 nM 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 100 nM increments

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