Associated products

Product	Description	Pack Size	Cat No.
ISOLATE II Genomic DNA Kit	Rapid isolation of high-quality genomic DNA from a wide variety of samples	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 and low abundance 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.

Email: tech@meridianlifescience.com

Trademark and licensing information

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Storage and Stability:
The SensiFAST Probe 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.

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 SensiFAST Probe 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:
Please refer to the material safety data sheet for further information.

Notes:
This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

SensiFAST™ Probe No-ROX Kit

Shipping: On dry/blue ice
Batch No.: See vial
Concentration: See vial

Catalog numbers
BIO-86005: 500 x 20 µL reactions: 5 x 1 mL
BIO-86020: 2000 x 20 µL reactions: 4 x 5 mL
BIO-86050: 5000 x 20 µL reactions: 10 x 5 mL

bioline meridian BIOSCIENCE

Store at –20 °C

Description

The SensiFAST™ Probe No-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR 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.

Kit components

Reagent	500 x 20 µL Reactions	2000 x 20 µL Reactions	5000 x 20 µL Reactions
SensiFAST Probe No-ROX mix (2x)	5 x 1 mL	4 x 5 mL	10 x 5 mL

Instrument compatibility

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

Manufacturer	Model
Bio-Rad	iCycler®, iQ™5, MyiQ™, Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384
Cepheid	SmartCycler™
Qiagen	Rotor-Gene™ 3000 & 6000
Eppendorf	Mastercycler® ep realplex
Roche	LightCycler® 480
Techne	Quantica®
BMS	Mic
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 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.

- 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 80-200 bp, and should not exceed 300 bp
- final primer concentration of 400 nM is suitable for most Probe-based reactions, however to determine the optimal concentration we recommend titrating in the range 0.2-1 µM. The forward and reverse primers concentration should be equimolar
- a final probe concentration of 100 nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration
  Note: Multiplex real-time PCR probe concentrations in excess of 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 must 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 1 µg of complex (e.g. eukaryotic) genomic DNA in a single PCR; we recommend using the Bioline ISOLATE II Genomic DNA Kit (BIO-52066) 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) for reverse transcription of the purified RNA; for high yield and purity of RNA, use Bioline ISOLATE II RNA Mini Kit (BIO-52072).

$MgCl_2$: 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) reaction, 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 20 µL final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
2x SensiFAST Probe No-ROX Mix	10 µL	1x
10 µM Forward Primer	0.8 µL	400 nM
10 µM Reverse Primer	0.8 µL	400 nM
10 µM Probe	0.2 µL	100 nM
Template	up to 8.2 µL	
H_2O	As required	

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.

Suggested thermal cycling conditions

The real-time PCR conditions, in the table below, are suitable for the SensiFAST Probe No-ROX Kit with the amplicons of up to 200 bp. These cycling parameters have been optimized on a number of platforms, however they can be varied to suit different machine-specific protocols.

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

***2 min for cDNA, up to 5min for genomic DNA**
****Up to 50s may be necessary for multiplexing with more than two 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 No-ROX is activated for 2 min 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 300 nM and 1 µM and probe concentration of 100 nM
	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
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling

Troubleshooting guide (Continued)

Problem	Possible Cause	Recommendation
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 300 nM and 1 µM and probe concentration of 100 nM
	Primer annealing/extension temperature(s) too low	Due to the high ionic strength of SensiFAST Probe 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 2 min 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 300 nM and 1 µM and probe concentration of 100 nM
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer/probe concentration too low	Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM
	Suboptimal design of primers/ probe	Use primer/probe design software or validated assays. Test primer/probe on a control template