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: mbi.tech@meridianlifescience.com

Trademark and licensing information

1).Trademarks: SensiFAST™ (Bioline Reagents Ltd), RotorGene™ (Qiagen), TaqMan® (ABI)

Bioline USA Inc.
USA

Tel: +1 901.382.8716
Fax: +1 901.382.0027

Storage and Stability:
The SensiFAST Probe Direct SuperMix 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 Direct SuperMix 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 research and/or further manufacturing use.

SensiFAST™ Probe Direct SuperMix

Shipping: On dry/blue ice

Batch No.: See vial

Concentration: See vial

Catalog numbers

BIO-86105: 500 x 20 µL reactions: 5 x 1 mL

BIO-86120: 2000 x 20 µL reactions: 4 x 5 mL

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Store at –20°C

Description

SensiFAST™ Probe Direct SuperMix has been designed for highly accurate, reproducible assay results in the presence of inhibitors, making it ideal for amplification directly from human and animal blood samples and, more generally biofluids containing PCR inhibitors. The kit has been formulated for use with probe-detection technology, such as TaqMan®. A combination of the latest advances in buffer chemistry and PCR enhancers, together with a hot-start DNA polymerase, ensures that the SensiFAST Probe Direct SuperMix delivers fast, highly-specific and ultra-sensitive real-time PCR.

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

Kit components

Reagent	500 x 20 µL Reactions	2000 x 20 µL Reactions
SensiFAST Probe Direct SuperMix (2x)	5 x 1 mL	4 x 5 mL

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.

Samples containing debris or particulate can interfere with the fluorescence detection of the signal. We therefore suggest using top reading instruments, or to dilute the sample if a bottom reading instrument is used (e.g. RotorGene platforms).

Template:
SensiFAST Probe Direct SuperMix can be used for amplification from crude lysates or inhibitor-rich samples such as urine, cerebral spinal fluid (CSF) or blood.

Due to the high viscosity, pipette a minimum of 4 µL of undiluted or diluted blood. The maximum recommended final concentration of blood in the reaction will be dependent of the qPCR platform, this may vary between 5 and 20 %.

Some optimization may be required.

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 80-200 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

$MgCl_2$: The SensiFAST Probe Direct SuperMix 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.

PI-50545

Website: www.bioline.com/sensifast email: info@meridianlifescience.com

Procedure

Reaction mix composition: Prepare a PCR master mix. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
SensiFAST Probe Direct SuperMix	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	

Resuspend reactions with care to minimize the formation of bubbles. Centrifuge reaction plate at 1200 x g for 6 minutes at 4 °C.

Suggested thermal cycling conditions

The real-time PCR conditions, in the table below, are suitable for SensiFAST Probe Direct SuperMix 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	* 3 min	Polymerase activation
40	95°C 60°C	10 s **20-50 s	Denaturation Annealing/extension (acquire at end of step)

*** 3 min is the optimal denaturation/activation time for most of the samples. However, extending the denaturation time (e.g. for 2 additional minutes) can result in an increased release of template in the mix.**
**** Up to 50 s 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	Template is carrying a too high concentration of inhibitors	Prepare serial dilutions of the sample to identify the optimal concentration of template and inhibitors.
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used. Always run a control reaction in absence of inhibitors.
	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
	Primers/probe degraded	Use newly synthesized primers and probe
	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
	Interference of sample with fluorescence detection system	Samples containing debris or particulate can interfere with the fluorescence detection of the signal. We therefore suggest using top reading instruments, or to dilute the sample if a bottom reading instrument is used (e.g. RotorGene platforms). Additionally, the sample could interfere with the fluorophores used for probe detection. Consider using alternative fluorophores.

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 Direct SuperMix 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
	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. Pipet viscous materials slowly and carefully.
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument
Late amplification trace	Activation time too short	The reaction can be activated for up to 5 min at 95 °C before cycling
	Extension time too short	Increasing the extension time may be necessary for amplification products over 200 bp; 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
	Sample is inhibiting the reaction	Dilute the sample, using serial dilutions to identify optimal conditions
	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