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
SensiFAST™ Probe Direct SuperMix	Highly accurate, reproducible assay results in the presence of inhibitors	500 Reactions 2000 Reactions	BIO-86105 BIO-86120
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
Uracil DNA Glycosylase‡	Catalyzes the release of uracil from uracil-containing single-stranded or double-stranded DNA	500 Units	BIO-27044
Agarose	Molecular biology grade agarose	100 g 500 g	BIO-41026 BIO-41025

‡ Not available in the US

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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Storage and stability:
SensiFAST Probe dUTP Mix 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:
SensiFAST Probe dUTP Mix 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 or further manufacturing use only.

Trademarks:
SensiFAST (Bioline Reagents Ltd), TaqMan (ABI), Scorpion (Qiagen)

SensiFAST™ Probe dUTP Mix

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

Catalog numbers
BIO-86201: 500 x 20 µL reactions: 5 x 1 mL
BIO-86220: 2,000 x 20 µL reactions: 4 x 5 mL

Store at –20°C

meridian BIOSCIENCE™

Description

SensiFAST™ Probe dUTP Mix is a 2x mix that has been formulated for use with probe-detection technology, such as 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 dUTP Mix delivers fast, highly-specific and ultra-sensitive qPCR.

The SensiFAST Probe dUTP Mix contains dUTP making it compatible with uracil DNA glycosylase to minimize carry-over PCR contamination.

Kit components

Reagent	500 x 20 µL reactions	2,000 x 20 µL reactions
SensiFAST Probe dUTP Mix (2x)	5 x 1 mL	4 x 5 mL

Instrument compatibility

The SensiFAST Probe dUTP Mix has been optimized for use with all probe chemistries, including TaqMan, FRET, Scorpions and molecular beacon probes.

The SensiFAST Probe dUTP Mix can be used on all real-time PCR instruments.

General considerations

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).

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 qPCR 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 qPCR:

- 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 reactions. However, to determine the optimal concentration we recommend titrating in the range 0.2-1 µM
- use an equimolar primer concentration
- 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 RT-qPCR, probe concentrations in excess of 100 nM 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 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-qPCR, we recommend using the SensiFAST cDNA Synthesis Kit for reverse transcription of the purified RNA; for high yield and purity of RNA, use ISOLATE II RNA Mini Kit.

$MgCl_2$: The SensiFAST Probe dUTP Mix contains an optimized concentration of $MgCl_2$, it is not necessary to supplement the mix further.

PCR controls: Always include a no-template control (NTC) reaction, replacing the template with PCR-grade water.

Procedure

Reaction mix composition: Prepare an qPCR 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
2x SensiFAST Probe dUTP 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	
20 µL Final volume		

† *SensiFAST Probe dUTP Mix is formulated with dUTP. When dUTP is incorporated into the amplification products, the amplicons are susceptible to degradation by uracil DNA glycosylase (UNG) (not provided); this allows you to incorporate UNG into subsequent reactions to control possible carryover contamination.*

Sensitivity testing and Ct 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 Ct value is not an indication of good sensitivity, but rather an indication of speed.

Suggested thermal cycling conditions
The qPCR conditions, in the table below, are suitable for the SensiFAST Probe dUTP Mix 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	*2-5 min	Polymerase activation
40	95°C	5 s	Denaturation
	60°C	**20-50 s	Annealing/extension (acquire at end of step)

**2 min for cDNA, up to 5 min 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	Ensure SensiFAST Probe dUTP Mix is activated for a minimum of 2 min at 95 °C before cycling
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer/probe design	Use primer design software or validated primers/probes. Test assay on a control template
	Incorrect concentration of primers/probe	Use primer concentrations between 200 nM and 1 µM. Probe concentration should be at least 2-fold lower than the primer concentration
	Primers/probe degraded	Use newly synthesized primers and/or probe
	Template contaminated with too many qPCR inhibitors	Further dilute template before qPCR or repurify template
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing time, increase cycle number, reduce annealing temperature
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Variability between replicates	Error in reaction set-up	Prepare large volume master mix
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument

Troubleshooting guide (Continued)

Problem	Possible Cause	Recommendation
Non-specific amplification product AND Primer-dimers	Suboptimal primer/probe design	Redesign primers and/or probe using appropriate software, or use validated primers/probes
	Primer/probe concentration too high	Test dilution series of primer/probe concentrations until primer-dimer/non-specific amplification products disappear
	Primer/probe concentration too low	Use primer concentration between 200 nM and 1 µM and probe concentration at least 2 fold lower
	Annealing/extension temperature too low	Increase annealing/extension temperature up to 65 °C or 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
	Carry-over PCR contamination	Incubate with uracil DNA glycosylase to degrade the carry-over DNA
	Extension time too long	Reduce extension time to determine whether non-specific products are reduced
Late amplification trace	Inefficient reverse transcription	Extend reverse transcription time up to 20 min and/or increase the temperature up to 48 °C
	Activation time too short	The reaction can be activated for up to 5 min at 95 °C before cycling
	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 primer/probe design	Redesign primers/probe using appropriate software, or use validated primers
	Primer/probe concentration too low	Increase concentration of primers in 100 nM increments and probe concentration in increments at least 2 fold lower than that of the primer
PCR efficiency below 90%	Extension time too short	Increase extension time
	Primer concentration too low	Increase concentration of primers in 100 nM increments
	Suboptimal primer/probe design	Redesign primer/probe using appropriate software or use validated primer/probe
PCR efficiency above 110%	Template is degraded or contains PCR inhibitors	Re-isolate template from sample material, or use freshly prepared template dilution, or purify template and resuspend it in water
	Non specific amplification and/or primer-dimers	Use 4% agarose gel electrophoresis to confirm presence of non-specific amplification products. See above for preventing/removing non-specific products