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

Licensing information

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SensiFAST™ SYBR® & Fluorescein Kit

Storage and stability:
SensiFAST SYBR® & Fluorescein 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:
SensiFAST SYBR® & Fluorescein Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity and absence of nuclease contamination and 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), SYBR (Molecular Probes), LightCycler (Roche), StepOne (ABI), RotorGene (Qiagen), LightCycler, ROX (Roche). iCycler MyiQ, IQ (Bio-Rad).

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

Catalog numbers
BIO-96005: 500 x 20 µL reactions: 5 x 1 mL
BIO-96020: 2000 x 20 µL reactions: 4 x 5 mL

Store at –20°C

meridian BIOSCIENCE™

Description

SensiFAST™ SYBR® & Fluorescein Kit uses a combination of the latest advances in buffer chemistry and enhancers, together with an antibody-mediated hot-start DNA polymerase system, to ensure fast, highly-specific and ultra-sensitive real-time PCR (qPCR). The kit has been validated on several Bio-Rad real-time PCR instruments.

For ease-of-use and added convenience, SensiFAST SYBR® & Fluorescein is provided as a 2x master mix containing all the components necessary for qPCR, including the SYBR® Green I dye, dNTPs, stabilisers and enhancers. The kit consists of a ready-to-use premix, only primers and template need to be added.

Kit components

Reagent	500 x 20 µL reactions	2000 x 20 µL reactions
SensiFAST™ SYBR® & Fluorescein mix (2x)	5 x 1 mL	4 x 5 mL

Instrument compatibility

SensiFAST SYBR® & Fluorescein Kit has been optimized for use in SYBR® Green-based qPCR on the real-time PCR instruments listed in the following compatibility table, each of these instruments having the capacity to analyze the qPCR data with the passive reference signal either on or off. The kit is also compatible with several instruments that do not require the use of ROX, such as the Mic (BMS), Qiagen Rotor-Gene™ 6000, the Bio-Rad CFX96 or the Roche LightCycler® 480.

Manufacturer	Model
Bio-Rad	iCycler®, MyiQ™, iQ®5

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: The specific amplification, yield and overall efficiency of any qPCR can be critically affected by the sequence and concentration of the 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
- optimal amplicon length should be 80-200 bp, and should not exceed 400 bp
- final primer concentration of 400 nM is suitable for most SYBR® -Green based reactions, however to determine the optimal concentration we recommend titrating in the range 0.1-1 µM. The forward and reverse primers concentration should be equimolar
- when amplifying from cDNA, use of intron spanning primers is preferable, 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. In addition, 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 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-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 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.

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-qPCR, set up a no-RT control as well as an NTC for the PCR.

Optional Fluorescein well-factor correction: SYBR® Fluorescein Kit is premixed with fluorescein, so that fluorescence emitted by fluorescein can be optionally detected on certain real-time instruments. If your real-time instrument has the capability of using fluorescein and you wish to use this option, then this option must be selected by the user in the software.

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.

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

Reagent	Volume	Final concentration
2x SensiFAST SYBR® & Fluorescein Mix	10 μL	1x
10μM Forward Primer	0.8 μL	400 nM
10μM Reverse Primer	0.8 μL	400 nM
Template	up to 8.4 μL	
H_2O	As required	

Suggested qPCR conditions: The following qPCR conditions are suitable for the SensiFAST SYBR® Fluorescein Kit with the amplicons of up to 200 bp. However, the cycling conditions can be varied to suit different machine-specific protocols. It is not recommended to use annealing temperatures below 60 °C or combined annealing/extension times longer than 30 seconds.

SensiFAST SYBR® Fluorescein Kit is compatible with either three-step or two-step cycling:

- **3-step cycling**

Cycles	Temp.	Time	Notes
1	*95 °C	*2 min	Polymerase activation
40	95 °C 60-65 °C 72 °C	5 s 10 s **5-20 s	Denaturation Annealing Extension (acquire at end of step)

***2 min for cDNA, 3 min for genomic DNA**
****Not recommended to extend beyond 20 seconds**

- **2-step cycling**

Cycles	Temp.	Time	Notes
1	*95 °C	*2 min	Polymerase activation
40	95 °C 60-65 °C	5 s **15-30 s	Denaturation Annealing/extension (acquire at end of step)

***2 min for cDNA, 3 min for genomic DNA**
****Not recommended to anneal/extend beyond 30 seconds**

Optional analysis: After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis.

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 SYBR® Fluorescein is activated for 2 min at 95 °C before cycling. For more complex templates such as genomic DNA, increase inactivation time up to 3 minutes.
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer design software or validated primers. Test primers on a control template
	Incorrect concentration of primers	Use primer concentration between 100 nM and 1 μM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
	Primers degraded	Use newly synthesized primers
	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

Troubleshooting guide (Continued)

Problem	Possible Cause	Recommendation
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Non-specific amplification product AND Primer-dimers	Suboptimal primer design	Redesign primers using appropriate software or use validated primers
	Primer concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primer concentration too low	Titrate primers in the concentration range of 100 nM - 1 μM
	Primer annealing/extension temperature(s) too low	Due to the high ionic strength of SensiFAST SYBR® Fluorescein 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 1 min and 3 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
	Template with high secondary structure	Increase reverse transcription reaction time up to 30 min Increase reverse transcription reaction temperature up to 45 °C
	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 concentration too low	Increase concentration of primer in 100 nM increments
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer concentration too low	Increase concentration of primer in 100 nM increments
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
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 melt analysis and 4% agarose gel electrophoresis to confirm presence of non-specific amplification products. See above for preventing/removing non-specific products