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 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

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Storage and Stability:
The SensiMix 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:
The SensiMix SYBR® & Fluorescein and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination prior to release.

Safety precautions:
Please refer to the material safety data sheet for further information.

Notes:
Research Use Only

SensiMix™ SYBR® & Fluorescein Kit

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

Catalog Numbers
QT615-02: 250 x 50 µlLreactions: 5 x 1.25 mL
QT615-05: 500 x 50 µL reactions: 10 x 1.25 mL
QT615-20: 2000 x 50 µL reactions: 40 x 1.25 mL

BIOLINE
A Meridian Life Science® Company

Store at –20 °C

Description

The SensiMix™ SYBR® & Fluorescein Kit is a high-performance product designed for superior sensitivity and specificity on real-time instruments, in which a fluorescein passive reference signal optionally used. The SensiMix SYBR® & Fluorescein Kit employs a hot-start DNA polymerase, for high PCR specificity and sensitivity. SensiMix SYBR® & Fluorescein is inactivated and possesses no polymerase activity during the reaction set-up, preventing non-specific amplification including primer-dimer formation.

For ease-of-use and added convenience SensiMix SYBR® & Fluorescein Kit is provided as a 2x mastermix containing all the components necessary for real-time PCR including the SYBR® Green I dye, dNTPs, stabilisers and enhancers. As a ready to use premix, only primers and template need to be added.

Kit Components

Reagent	250 x 50 µL reactions	500 x 50 µL reactions	2000 x 50 µL Rreactions
SensiMix™ SYBR® & Fluorescein (2X)	5 x 1.25 mL (6.25 mL)	10 x 1.25 mL (12.5 mL)	40 x 1.25 mL (50 mL)

Kit Compatibility

The 2X SensiMix SYBR® & Fluorescein Kit has been optimized for use in SYBR Green-based real-time PCR on the real-time instruments listed in the following compatibility table, each of these instruments having the capacity to analyze the real-time PCR 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 fluorescein, such as the BMS Mic, Qiagen (Corbett) Rotor-Gene™ 6000, Bio-Rad CFX96 or 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 PCR set-up, PCR amplification and any post-PCR gel analysis. It is essential that any amplified PCR product should not be opened in PCR set-up area.

Primers: the sequence and concentration of primer as well as the amplicon length can be critical for specific amplification, yield and overall efficiency of any real-time PCR. 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 60 °C
- optimal amplicon length should be 50-150 bp
- a final primer concentration of 250 nM is suitable for most PCR conditions, however to determine the optimal concentration we recommend a primer titration in the range of 0.1–1 µM
- use equimolar primer concentrations
- when amplifying from cDNA use gene-specific primers. If possible 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 Bioline ISOLATE II Genomic DNA Mini 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 Bioline 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 5mM.

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-PCR, set-up a no RT control as the 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 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™ SYBR® & Fluorescein	25 µL	1x
25 µM Forward Primer	0.5 µL	250 nM
25 µM Reverse Primer	0.5 µL	250 nM
H_20	Up to 45 µL	-
Template	5 µL	
50 µL Final volume		

Suggested Thermo-cycling conditions

The following PCR conditions are suitable for SensiMix SYBR® & Fluorescein Kit with a majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit customer 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 (SYBR®) Green or FAM.

Cycles	Temperature	Time	Notes
1	*95 °C	*10 min	Polymerase activation
40	95 °C 55-60 °C 72 °C	15 s 15 s 15 s	Temp. depends on the Tm of primers Acquire at end of step

***Non-variable parameter**

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	Make sure SensiMix is activated for 10 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 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 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 Product 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 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 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 the reaction is activated for 10 min 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 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 H_2O
	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

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@bioline.com