SensiMix™ SYBR & Fluorescein Kit

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

Catalog Numbers
QT615-02: 250 x 50µl reactions: 5 x 1.25ml
QT615-05: 500 x 50µl reactions: 10 x 1.25ml
QT615-20: 2000 x 50µl reactions: 40 x 1.25ml

Store at –20°C

Storage and Stability:
The SensiMix™ SYBR & Fluorescein Kit is shipped on Dry/ Blue Ice and all its components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended. Since SYBR® Green I is light sensitive, it is important to avoid prolonged exposure to light. When stored under optimum conditions, the reagents are stable for a minimum of 6 months from date of purchase.

Quality control:
Bioline operates under ISO 9001 Management System. 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:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Note:
For research use only

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 inactivate 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 50µl Reactions	500 x 50µl Reactions	2000 x 50µl Reactions
SensiMix™ SYBR & Fluorescein (2X)	5 x 1.25ml (6.25ml)	10 x 1.25ml (12.5ml)	40 x 1.25ml (50ml)
50mM $MgCl_2$	1 x 1ml	1 x 1ml	4 x 1ml

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 Qiagen (Corbett) 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 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 50-150bp
- a final primer concentration of 250nM is suitable for most PCR conditions, however to determine the optimal concentration we recommend a primer titration in the range of 0.1–1µM
- 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 1µg of complex (e.g. eukaryotic) genomic DNA in a single PCR. We recommend using the Bioline ISOLATE Genomic DNA Mini Kit (BIO-53021) 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 100ng cDNA per reaction, however it may be necessary to vary this amount. To perform a two-step RT-PCR, we recommend using the Bioline cDNA Synthesis Kit (BIO-65026) for reverse transcription of the purified RNA. For high yield and purity of RNA, use the Bioline ISOLATE RNA Mini Kit (BIO-54042)

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM. 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 (*see notice to purchaser No. 5 in Trademark and Licensing Information*).

Procedure

Reaction mix composition: Prepare a PCR master mix. The volumes given below are based on a standard 50µl final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
2x SensiMix™ SYBR & Fluorescein	25µl	1x
25µM Forward Primer	0.5µl	250nM
25µM Reverse Primer	0.5µl	250nM
$H_2$0	Up to 45µl	-
Template	5µl	
	50µl 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	*10min	Polymerase activation
40	95°C 55-60°C 72°C	15s 15s 15s	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 10min 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 100nM and 1µM
	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

Website: www.bioline.com/sensimix email: info@bioline.com

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 100nM - 1μM
	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 10min 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 30min 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 100nM increments
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer concentration too low	Increase concentration of primer in 100nM 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

Associated Products

Product	Description	Pack Size	Cat No.
ISOLATE Genomic DNA Mini kit	Rapid isolation of DNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52031 BIO-52032 BIO-52033
ISOLATE Plant DNA Mini kit	Rapid isolation of DNA from a variety of plant samples	10 Preps 50 Preps 250 Preps	BIO-52034 BIO-52035 BIO-52036
ISOLATE RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52039 BIO-52040 BIO-52041
ISOLATE Plant RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of plant samples	10 Preps 50 Preps 250 Preps	BIO-52042 BIO-52043 BIO-52044
TRIsure™	Quick isolation of high-quality RNA from a variety of sources for subsequent use in cDNA synthesis	100ml 200ml	BIO-38032 BIO-38033
cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length cDNA from RNA	30 Reactions 100 Reactions	BIO-65025 BIO-65026
Agarose	Molecular biology grade agarose	100g 500g	BIO-41026 BIO-41025
PCR Water	Ultra-pure (18.2MΩ) molecular biology grade water	10 x 10ml	BIO-37080
DEPC-treated Water	Deionized, high-quality molecular grade water treated with DEPC. Ideal for use in all RNA work	10 x 10ml 1 Liter	BIO-38030 BIO-38031

TRADEMARK AND LICENSING INFORMATION
1).Trademarks: SensiMix™ (Bioline Reagents Ltd), SYBR® (Molecular Probes), ROX™, LightCycler™ (Roche), StepOne™ (ABI), RotorGene™ (Qiagen), LightCycler® (Roche). iCycler™ MyiQ™, IQ™ (Bio-Rad).

2). Purchase of this product includes limited right to use the supplied amount of SYBR® Green I Stain patented by Molecular Probes, Inc.

3) Notice to Purchaser: Limited License. Use of this product is covered by one or more of the following US patents: 5,079,352,5,789,224, 5,618,711, 6,127,155, 5,677,152 (claims 1 to 23 only), 5,773,258 (claims 1 and 6 only). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as the patented 5' Nuclease Process claims in US Patents Nos. 5,210,015 and 5,487,972) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

4) SensiMix products are manufactured by Bioline Reagents Ltd.

5) Notice to Purchaser: No rights are conveyed with respect to US patent 5,928,907

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