

SensiMix™ SYBR No-ROX One-Step Kit

Shipping: On Dry/Blue Ice	Catalog Numbers
Exp. Date: See vial	QT235-01: 25 x 50µl reactions: 1 x 625µl
Batch No.: See vial	QT235-02: 250 x 50µl reactions: 5 x 1.25ml
Concentration: see vial	QT235-05: 500 x 50µl reactions: 10 x 1.25ml
	QT235-20: 2000 x 50µl reactions: 40 x 1.25ml

Store at -20°C

Storage and Stability:

The SensiMix™ SYBR No-ROX One-Step Kit is shipped on Dry/Blue Ice. All kit 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 No-ROX One-Step Kit 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.



DATA SHEET

Description

The SensiMix™ SYBR No-ROX One Step Kit contains a ready-to-use reagent for real-time RT-PCR for use on various real-time instruments that do not need a passive reference signal for normalization of the data. The kit provides high-performance, reproducible first strand cDNA synthesis and subsequent SYBR Green-based real-time PCR assays, based a single protocol. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. For high specificity and sensitivity of the one-step RT-PCR, SensiMix SYBR No-ROX One-Step Kit combines a reverse transcriptase and a proprietary hot-start DNA polymerase. The hot-start DNA polymerase is inactivated and possesses no polymerase activity during the reaction set-up, preventing non-specific amplification including primer-dimer formation.

The SensiMix SYBR No-ROX One-Step Kit consists of a 2x SensiMix SYBR No-ROX One-Step mix, separate reverse transcriptase and RiboSafe RNase Inhibitor.

Kit components

Reagent	25 x 50µl Reactions	250 x 50µl Reactions	500 x 50µl Reactions	2000 x 50µl Reactions
SensiMix™ SYBR No-ROX One-Step (2x)	1 x 625µl	5 x 1.25ml	10 x 1.25ml	40 x 1.25ml
50mM MgCl ₂	1 x 1ml	1 x 1ml	1 x 1ml	4 x 1ml
RNase Inhibitor (10U/µl)	1 x 25µl	1 x 250µl	1 x 500µl	4 x 500µl
100x Reverse Transcriptase	1 x 12.5µl	1 x 125µl	1 x 250µl	1 x 1ml
DEPC-H ₂ O	1 x 1.8ml	2 x 1.8ml	4 x 1.8ml	16 x 1.8ml

Kit compatibility

The 2x SensiMix SYBR No-ROX One-Step Kit is premixed with SYBR Green I dye and is compatible with real-time instruments that do not need a passive reference signal for normalization of the data. The SensiMix SYBR No-ROX One-Step Kit is optimized for use on the real-time instruments listed in the following compatibility table.

Manufacturer	Model
Bio-Rad	Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384
Cepheid	SmartCycler™
Corbett	Rotor-Gene™ 3000 & 6000
Eppendorf	Realplex
Roche	LightCycler® 480
Techne	Quantica®

General considerations

When handling RNA it is important to use RNase-free plasticware and reagents. We also recommend performing RNA work in an isolated area.

To help prevent any carry-over of DNA contamination, we recommend that separate areas in the laboratory are maintained for PCR 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 sequence and concentration of the primers as well as the amplicon length can be critical for correct amplification, yield and overall efficiency of any real-time RT-PCR. Therefore we strongly recommend taking the following into consideration when designing and running your RT-PCR:

- 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 (T_m) of approximately 60°C
- optimal amplicon length should be 50-150bp, and should not exceed 400bp
- 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 0.1-1µM
- use equimolar primer concentrations
- use intron-spanning primers to avoid amplification from genomic DNA

Template: It is important for both reverse transcription and subsequent PCR, that the RNA template is intact and devoid of contaminating inhibitors. For high-purity RNA, we recommend using the Bioline ISOLATE RNA Mini Kit (BIO-52043) and resuspending the RNA in DEPC-treated Water (BIO-38030), to avoid any RNase-mediated degradation prior to use in RT-PCR.

The recommended amount of template for one-step RT-PCR is dependent upon the type of RNA used. The following should be considered when using total RNA and isolated mRNA:

- **total RNA:** purified total RNA can be used in the range from 1pg to 1µg per 50µl reaction
- **mRNA:** purified mRNA can be used from 0.5pg per 50µl reaction

MgCl₂: The MgCl₂ concentration in the 1x reaction mix is 3mM, which is optimal for both Reverse Transcriptase and polymerase in the majority of real-time RT-PCR conditions. We recommend, if necessary, titrating MgCl₂ to a maximum of 5mM.

RT-PCR Controls: It is important to detect the presence of contaminating genomic DNA that may affect the reliability of the data. Always include a no-template control, replacing the template with PCR-grade water and a no-Reverse Transcriptase control to ensure the RNA sample is not contaminated with DNA.

Optional ROX: The SensiMix SYBR No-ROX Kit is premixed with ROX (5-carboxy-X-rhodamine, succinimidyl ester), so that where necessary, ROX fluorescence can be optionally detected on certain real-time instruments. If your real-time instrument has the capability of using ROX 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 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 No-ROX One-Step	25µl	1x
25µM Forward Primer	0.5µl	250nM
25µM Reverse Primer	0.5µl	250nM
100x Reverse Transcriptase	0.5µl	1x
10U/µl RiboSafe RNase Inhibitor	1µl	0.2U/µl
DEPC-H ₂ O	Up to 45µl	-
Template	5µl	
50µl Final Volume		

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 of between 100nM and 1µM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution. We recommend using the Isolate RNA kits for template preparation and DEPC-treated water for resuspension or dilution of the template Verify the integrity of the RNA using agarose electrophoresis
	Primers degraded	Use newly synthesized primers
	Template contaminated with inhibitors	Further dilute template before RT-PCR or purify template and resuspend it in DEPC-treated water
	Template concentration too low	Increase concentration used
No amplification trace AND Product on agarose gel	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number, reduce annealing temperature
	Error in instrument setup	Check that the acquisition settings are correct during cycling

Suggested RT-PCR conditions: The cycling conditions can be varied to suit customer's or machine-specific protocols. The critical step of the RT-PCR is the 10-minute polymerase activation at 95°C. The detection channel on the real-time instrument should be set to (SYBR) green or FAM.

The following PCR cycling conditions are suitable for SensiMix SYBR No-ROX One-Step with a majority of samples and real-time PCR instruments.

• 3-step cycling

Cycles	Temperature	Time	Notes
1	45°C	10min	Reverse transcription
1	*95°C	*10min	Polymerase activation
40	95°C	15s	Temperature depends on the T _m of primers Acquire at end of step
	55-60°C	15s	
	72°C	15s	

*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 (Continued)

Problem	Possible Cause	Recommendation
Non-specific amplification product AND Primer-dimers	Suboptimal primer design	Redesign primers using appropriate software or use pre-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 - 1 μ M
	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 pre-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 pre-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 ₂ O
	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

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
Tetro cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length cDNA from RNA	30 Reactions 100 Reactions	BIO-65042 BIO-65043
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	Deionised, high-quality molecular grade water treated with DEPC. Ideal for use in all RNA work	10 x 10ml 1 Litre	BIO-38030 BIO-38031

TRADEMARK AND LICENSING INFORMATION

1) Trademarks: SensiMix™ (Bioline Reagents Ltd), SYBR® (Molecular Probes), iCycler™ MyiQ5™, Opticon™, Chromo4™, MiniOpticon™, (Bio-Rad), LightCycler® (Roche), StepOne™ (ABI), SmartCycler™ (CEPheid), RotorGene™ (Qiagen), RealPlex™ (Eppendorf), Quantica™ (Techne), MX4000 (Stratagene).

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 may be covered by one or more of the following US patents: 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 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

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium