SensiFAST™ Probe No-ROX One-Step Kit

Shipping: On Dry/Blue Ice

Exp. Date: See vial

Batch No.: See vial

Concentration: see vial

Catalog Numbers

BIO-76001: 100 x 20µl reactions: 1 x 1ml

BIO-76005: 500 x 20µl reactions: 5 x 1ml

BIOLINE

A Meridian Life Science® Company

Store at –20°C

Storage and Stability:
The SensiFAST Probe 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. 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 SensiFAST Probe 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.

Safety Precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Notes:
For research use only.

Description

The SensiFAST™ Probe No-ROX One-Step Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The kit is formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. A combination of the latest advances in buffer chemistry together with a reverse transcriptase and hot-start DNA polymerase system ensures that SensiFAST Probe No-ROX One-Step Kit produces fast, highly-specific and ultra-sensitive one-step RT-qPCR.

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

Kit components

Reagent	100 x 20µl Reactions	500 x 20µl Reactions
SensiFAST™ Probe No-ROX One-Step mix (2x)	1 x 1ml	5 x 1ml
RiboSafe RNase Inhibitor	1 x 40µl	1 x 200µl
Reverse transcriptase	1 x 20µl	1 x 100µl
DEPC-H_2O	1 x 1.8ml	2 x 1.8ml

Instrument compatibility

The SensiFAST Probe No-ROX One-Step Kit has been optimized for use with all probe chemistries, including TaqMan, FRET, Scorpions and molecular beacon probes.

The SensiFAST Probe No-ROX One-Step Kit can be used on all real-time PCR instruments.

General considerations

When handling RNA, it is important to use RNase-free plasticware and reagents. We also recommend performing RNA work in an RNase-free area. 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 DNA product are not opened in the reaction set-up area.

Primers and probe: These guidelines refer to the use of dual-labeled probes. Please refer to the relevant literature when using other probe types. The sequence and concentration of the probe and primers, as well as amplicon length, can be critical for specific amplification, yield and overall efficiency of any RT-qPCR.

We strongly recommend taking the following points into consideration when designing and running your RT-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 80-200bp, and should not exceed 400bp
- final primer concentration of 400nM is suitable for most probe reactions. However, to determine the optimal concentration we recommend titrating in the range 0.2-1µM
- use an equimolar primer concentration
- a final probe concentration of 100nM 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 100nM can result in cross-channel fluorescence
- where possible, use intron-spanning primers to avoid amplification from genomic DNA

Template: It is important that the RNA template is intact and devoid of DNA or contaminating inhibitors of both reverse transcription and PCR. For high purity RNA, we recommend using the Bioline ISOLATE RNA Mini Kit (BIO-52043). RNA stocks and dilutions should be made in DEPC-treated Water (BIO-38030) to avoid any RNase-mediated degradation.

The recommended amount of template for one-step RT-qPCR is dependent upon the type of RNA used:

- **total RNA:** purified total RNA can be used in the range from 1pg to 1µg per 20µl reaction
- **mRNA:** purified mRNA can be used from 0.01pg per 20µl reaction

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of RT-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.

RT-PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-RT control, by omitting the reverse transcriptase from the reaction.

Procedure

Reaction mix composition: Prepare an RT-PCR mastermix. The volumes given below are based on a standard 20µl final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
2x SensiFAST Probe No-ROX One-Step Mix	10µl	1x
10µM Forward Primer	0.8µl	400nM
10µM Reverse Primer	0.8µl	400nM
10µM Probe	0.2µl	100nM
Reverse transcriptase	0.2µl	-
RiboSafe RNase Inhibitor	0.4µl	-
H_2O	up to 16µl	
Template	4µl	
20µl Final volume		

Suggested RT-qPCR conditions: The following RT-qPCR conditions are suitable for the SensiFAST Probe No-ROX One-Step Kit with the majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit different probe-based reactions or machine-specific protocols. The detection channel on the real-time instrument should be set to acquire at the appropriate wavelength(s). We recommend using the following cycling conditions for optimal results:

- **Cycling for dual-labeled probes**

Cycles	Temperature	Time	Notes
1	45°C	10min	Reverse transcription
1	95°C	2min	Polymerase activation
40	95°C 60°C	5s 20s	Denaturation Annealing/extension (acquire at end of step)

RT-qPCR optimization: The following optimization may be necessary to improve the efficiency of some reactions, such as multiplexing with more than 2 probes, or if the target amplicon is longer than 200bp.

- The reverse transcription reaction time can be extended up to 20 minutes and/or the temperature can be increased up to 48°C
- The annealing/extension time can be extended up to 60 seconds and/or the temperature can be increased up to 65°C

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	Ensure SensiFAST Probe No-ROX One-Step mix is activated for a minimum of 2min 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 200nM and 1µM. Probe concentration should be at least 2-fold lower than the primer concentration
	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 RNA using agarose gel electrophoresis
	Primers/probe degraded	Use newly synthesized primers and/or probe
	Template contaminated with RT-PCR inhibitors	Further dilute template before RT-PCR or purify template and resuspend it in DEPC-treated water
	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

Troubleshooting Guide (Continued)

Problem	Possible Cause	Recommendation
Non-specific amplification product AND Primer-dimers	Inefficient reverse transcription	Extend reverse transcription time up to 20min and/or increase the temperature up to 48°C
	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 200nM and 1µM 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
	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
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument
Late amplification trace	Inefficient reverse transcription	Extend reverse transcription time up to 20min and/or increase the temperature up to 48°C
	Activation time too short	Ensure SensiFAST Probe No-ROX One-Step mix is activated for a minimum of 1min 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 is degraded	Re-isolate template from sample material or use freshly prepared template dilution
	Suboptimal primer/probe design	Redesign primers/probe using appropriate software, or use validated primers
	Primer/probe concentration too low	Increase concentration of primers in 100nM increments and probe concentration in increments at least 2 fold lower than that of the primer
	RNase contamination	Ensure RNase inhibitor is added before addition of template
PCR efficiency below 90%	Extension time too short	Increase extension time
	Primer concentration too low	Increase concentration of primers in 100nM 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

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

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

Trademark and Licensing Information

Bioline Ltd
UNITED KINGDOM
Tel: +44(0)20 8830 5300
Fax: +44 (0)20 8452 2822

Bioline USA Inc.
USA
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Bioline GmbH
GERMANY
Tel: +49(0)33 7168 1229
Fax: +49 (0)337168 1244

Bioline (Aust) Pty. Ltd
AUSTRALIA
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763