

SensiMix™ Probe One-Step Kit

Shipping: On Dry/Blue Ice	Catalog numbers
Exp. Date: See vial	QT725-01: 25 x 50µl reactions: 1 x 625µl
Batch No.: See vial	QT725-02: 250 x 50µl reactions: 5 x 1.25ml
Concentration: see vial	QT725-05: 500 x 50µl reactions: 10 x 1.25ml
	QT725-20: 2000 x 50µl reactions: 40 x 1.25ml



Store at -20°C

Storage and stability:

The SensiMix™ Probe 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 SensiMix Probe 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.

Description

The SensiMix™ Probe One Step Kit contains a ready-to-use reagent for probe-based real-time RT-PCR for use on all real-time instruments. The kit has been formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. The SensiMix Probe One-Step Kit provides high-performance, reproducible first strand cDNA synthesis and subsequent real-time PCR assays, using a single protocol. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. The kit combines a reverse transcriptase and a hot-start DNA polymerase, for high specificity and sensitivity of the one-step RT-PCR. SensiMix 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 Probe One-Step Kit is provided as a 2x mastermix containing all the components necessary for reverse transcription and real-time PCR including dNTPs and stabilisers. As a ready-to-use premix, only primers and template need to be added. In addition, SensiMix Probe One-Step Kit includes the highly efficient RiboSafe RNase inhibitor, which prevents enzymatic degradation of the RNA. In addition a separate tube of ROX is provided for optional use.

Kit components

Reagent	25 x 50µl Reactions	250 x 50µl Reactions	500 x 50µl Reactions	2000 x 50µl Reactions
SensiMix™ Probe 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
25µM ROX dye	1 x 25µl	1 x 250µl	1 x 500µl	4 x 500µl
DEPC-H ₂ O	1 x 1.8ml	2 x 1.8ml	4 x 1.8ml	16 x 1.8ml

Kit compatibility

The SensiMix Probe One-Step Kit has been optimized for use with all widely used probe chemistries, including TaqMan, FRET, Scorpions and molecular beacon probes. In addition, the SensiMix Probe 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 isolated area. 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 tubes containing amplified PCR product are not opened in the PCR set-up area.

Primers and probe: These guidelines refer to the use of TaqMan 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 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 (T_m) of approximately 58-60°C. The T_m of the probe should be approximately 10°C higher than that of the primers
- optimal amplicon length should be 80-150bp, 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 of 0.3-1µM
- use an equimolar primer concentration
- final probe concentration of 200nM is suitable for most applications. We recommend that the final probe concentration is lower than the primer concentration
- if possible, use intron-spanning primers to avoid amplification from genomic DNA

Template: It is important that the RNA template is intact and devoid of contaminating inhibitors for both reverse transcription and subsequent PCR. For high purity RNA, we recommend using the Bioline ISOLATE RNA Mini Kit (BIO-52043). Prior to use in RT-PCR, RNA suspensions 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-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 SensiTaq in the majority of real-time RT-PCR conditions. If necessary, we recommend 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.

Optional ROX : Reaction-independent ROX fluorescence can be measured on the real-time instruments listed below, to normalize the reporter-dye signal during PCR. 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. Sensimix Probe One-Step Kit is supplied with a separate tube of ROX at 25 μ M, which can be added as indicated in the table per 50 μ l reaction.

Manufacturer	Model	ROX volume 50 μ L reaction	Final ROX concentration
ABI	7000, 7300, 7700, 7900, 7900HT and StepOne™	1.0 μ l	500nM
	7500 and StepOne™ Plus	0.1 μ l	50nM
Stratagene	Mx4000™, Mx3000P™, Mx3005™	0.1 μ l	50nM

Table 1.

Procedure

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

Reagent	Volume	Final concentration
2x SensiMix ProbeOne-Step	25 μ l	1x
10 μ M Forward Primer	2 μ l	400nM
10 μ M Reverse Primer	2 μ l	400nM
10 μ M Probe	1 μ l	200nM
10 μ M ROX (see Table 1.)	-	-
10U/ μ l RiboSafe RNase Inhibitor	1 μ l	0.2U/ μ l
DEPC-H ₂ O	Up to 45 μ l	-
Template	5 μ l	
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 primers/probe design	Use primers/probe design software or validated primers. Test primers/probe on a control template
	Incorrect concentration of primers/probe	Use primer concentration between 300nM and 1 μ M and probe concentration at least 2-fold lower
	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
	Primers/probe degraded	Use newly synthesized primers/probe
	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
Cycling conditions not optimal	Increase extension/annealing times, increase cycle number, reduce annealing temperature	

Suggested RT-PCR conditions: The following RT-PCR conditions are suitable for SensiMix Probe One-Step Kit with a 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 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 acquire at the appropriate wavelength(s).

Standard cycling for TaqMan probes

Cycles	Temperature	Time	Notes
1	42°C	10min	Reverse transcription
1	*95°C	*10min	Polymerase activation
40	95°C	10s	Acquire at end of step
	60°C	60s	

*Non-variable parameter

Troubleshooting guide (continued)

Problem	Possible cause	Recommendation
No amplification trace AND Product on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Non-specific amplification product AND Primer-dimers	Suboptimal primers/probe design	Redesign primers using appropriate software or use validated primers
	Primers/probe concentration too high	Test dilution series of primers/probe concentrations until primer-dimer/non-specific amplification products disappear
	Primers/probe concentration too low	Use primer concentration between 300nM and 1µM and probe concentration at least 2-fold lower
	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/probe	Redesign primers/probe using appropriate software or use validated primers/probe
	Primers/probe concentration too low	Increase concentration of primer in 100nM increments, adjust probe concentration to half the primer concentration
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primers/probe concentration too low	Increase concentration of primer in 100nM increments, adjust probe concentration to half the primer concentration
	Suboptimal design of primers/probe	Redesign primers/probe using appropriate software or use validated primers/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 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

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
 UK: +44 (0) 20 8830 5300
 Germany: +49 (0) 3371 681 229
 USA: +1 508 880 8990
 Australia: +61 (0)2 9209 4180
 All Others Nations: Local distributor or 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

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3) SensiMix products are manufactured by Bioline Reagents Ltd.

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

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