SensiFAST™ Probe No-ROX One-Step Kit

Bioline

Abstract

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 real-time RT-PCR.

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

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Guidelines

Kit components

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

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 real-time RT-PCR.

We strongly recommend taking the following points into consideration when designing and running your real-time RT-PCR:

- use primer-design software, such as Primer3 (http:// frodo.wi.mit.edu/primer3/) or visual OMPTM (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 80-200 bp, and should not exceed 400 bp
- final primer concentration of 400 nM is suitable for most probe reactions. However, to determine the optimal concentration we recommend titrating in the range 0.2-1 µM
- use an equimolar primer concentration
- a final probe concentration of 100 nM 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 real-time RT-PCR, probe concentrations in excess of 100 nM 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 II RNA Mini Kit (BIO-52073). RNA stocks and dilutions should be made in DEPC-treated water

to avoid any RNase-mediated degradation.

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

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

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3 mM. In the majority of real-time RT-PCR 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 5 mM.

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 reaction, by omitting the reverse transcriptase from the reaction.

Troubleshooting Guide:

See the Bioline full documentation for detailed troubleshooting instructions.

http://www.bioline.com/us/downloads/dl/file/id/3303/sensifast_probe_no_rox_one_step_kit_manual.pdf

Materials

SensiFAST™ Probe No-ROX One-Step Kit BIO-76001 by Bioline

Protocol

Reaction mix composition

Step 1.

Prepare an real-time RT-PCR mastermix. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly.

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

Sensitivity testing and Ct values

Step 2.

When comparing SensiFAST with a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early C_t value is not an indication of good sensitivity, but rather an indication of speed.

Suggested RT-qPCR conditions

Step 3.

The following real-time RT-PCR 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	Temp.	Time	Notes
1	45°C	10 min	Reverse transcription
1	95°C	2 min	Polymerase activation
40	95°C 60°C	5s 20 s	Denaturation Annealing/extension (acquire at end of step)

Real-Time RT-PCR optimization

Step 4.

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

- 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