SensiFAST™ Probe No-ROX Kit

Bioline

Abstract

The SensiFAST™ Probe No-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR instruments. The kit has been formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. A combination of the latest advances in buffer chemistry and PCR enhancers, together with a hot-start DNA polymerase, ensures that the SensiFAST Probe Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.

SensiFAST Probe is provided as a 2x mastermix containing all the components necessary for real-time PCR, including dNTPs, stabilizers and enhancers.

Citation: Bioline SensiFAST™ Probe No-ROX Kit. **protocols.io**
dx.doi.org/10.17504/protocols.io.fyxbpxn
Published: 24 Nov 2016

Guidelines

Kit components

Reagent	200 x 20 µL Reactions	500 x 20 µL Reactions	2000 x 20 µL Reactions
SensiFAST Probe No-ROX mix (2x)	2 x 1 mL	5 x 1 mL	4 x 5 mL

Instrument compatibility

The SensiFAST Probe No-ROX Kit is compatible with real-time PCR instruments that do not need a passive reference signal for normalization of the data. The SensiFAST Probe No-ROX Kit has been optimized for use on the real-time PCR instruments listed in the following compatibility table.

Manufacturer	Model
Bio-Rad	iCycler®, iQTM5, MyiQTM, Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384
Cepheid	SmartCycler™
Qiagen	Rotor-Gene™ 3000 & 6000
Eppendorf	Mastercycler® ep realplex
Roche	LightCycler® 480
Techne	Quantica®
BMS	Mic
Takara	Thermal Cycler Dice® (TP800)

General considerations

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 PCR product are not opened in the PCR set-up area.

Primers and probe: These guidelines refer to the design and set-up of TaqMan probe-based PCR. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the probes and primers, as well as by the amplicon length.

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

- 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 80-200 bp, and should not exceed 300 bp
- final primer concentration of 400 nM is suitable for most Probe-based reactions, however to determine the optimal concentration we recommend titrating in the range 0.2-1 µM. The forward and reverse primers concentration should be equimolar
- a final probe concentration of 100 nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration

Note: Multiplex real-time PCR probe concentrations in excess of 100nM, can result in cross- channel fluorescence

Template: It is important that the DNA template is suitable for use in PCR in terms of purity and concentration. In addition, the template must 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 points should be considered when using genomic DNA and cDNA templates:

- Genomic DNA: use up to 1 µg of complex (e.g. eukaryotic) genomic DNA in a single PCR; we recommend using the Bioline ISOLATE II Genomic DNA Kit (BIO-52066) 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 100 ng cDNA per reaction, however it may be necessary to vary this amount; to perform a two-step RT-PCR, we recommend using the SensiFAST cDNA Synthesis Kit (BIO-65053) for reverse transcription of the purified RNA; for high yield and purity of RNA, use Bioline ISOLATE II RNA Mini Kit (BIO-52072).

$MgCl_2$: The SensiFAST Probe mix contains an optimized concentration of $MgCl_2$, it is not necessary to supplement the mix further.

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) reaction, replacing the template with PCR-grade water. When performing a two-step RT-PCR, set up a no-RT control as well as an NTC for the PCR.

Troubleshooting Guide:

See the Bioline full documentation for detailed troubleshooting instructions.

http://www.bioline.com/us/downloads/dl/file/id/2686/sensifast_probe_no_rox_kit_manual.pdf

Materials

SensiFAST™ Probe No-ROX Kit BIO-86002 by Bioline

Protocol

Reaction mix composition

Step 1.

Prepare a 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 Mix	10µL	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
Template	up to 8.2 µL	
H_2O	As required	

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 thermal cycling conditions

Step 3.

The real-time PCR conditions, in the table below, are suitable for the SensiFAST Probe No-ROX Kit with the amplicons of up to 200 bp. These cycling parameters have been optimized on a number of platforms, however they can be varied to suit different machine-specific protocols.

Cycles	Temp.	Time	Notes
1	95°C	*2-5min	Polymerase activation
40	95°C 60°C	10s ** 20-50s	Denaturation Annealing/extension (acquire at end of step)

*2 min for cDNA, up to 5min for genomic DNA
**Up to 50s may be necessary for multiplexing with more than two probes