SensiFAST™ SYBR & Fluorescein Kit

Bioline

Abstract

The SensiFAST™ SYBR® & Fluorescein Kit uses a combination of the latest advances in buffer chemistry and enhancers, together with an antibody-mediated hot-start DNA polymerase system, to ensure fast, highly-specific and ultra-sensitive real-time PCR. The kit has been validated on several Bio-Rad real-time PCR instruments.

For ease-of-use and added convenience, SensiFAST SYBR® & Fluorescein is provided as a 2x mastermix containing all the components necessary for real-time PCR, including the SYBR® Green I dye, dNTPs, stabilisers and enhancers. The kit consists of a ready-to-use premix, only primers and template need to be added.

Citation: Bioline SensiFAST™ SYBR & Fluorescein Kit. **protocols.io**
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Guidelines

Kit components

Reagent	200 x 20µL reactions	500 x 20µL reactions	2000 x 20µL reactions
SensiFASTTM SYBR® & Fluorescein mix (2x)	2 x 1 mL	5 x 1 mL	4 x 5 mL

Instrument compatibility

SensiFAST SYBR® & Fluorescein Kit has been optimized for use in SYBR® Green-based real-time PCR on the real-time PCR instruments listed in the following compatibility table, each of these instruments having the capacity to analyze the real-time PCR data with the passive reference signal either on or off. The kit is also compatible with several instruments that do not require the use of ROX, such as the Mic (BMS), Qiagen (Corbett) Rotor- Gene™ 6000, the Bio-Rad CFX96 or the Roche LightCycler® 480.

Manufacturer	Model
Bio-Rad	iCycler®, MyiQ™, iQ®5

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: The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the 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
- optimal amplicon length should be 80-200 bp, and should not exceed 400 bp

- final primer concentration of 400n M is suitable for most SYBR® -Green based reactions, however to determine the optimal concentration we recommend titrating in the range 0.1-1 µM. The forward and reverse primers concentration should be equimolar
- when amplifying from cDNA, use of intron spanning primers is preferable, to avoid amplification from genomic DNA

Template: it is important that the DNA template is suitable for use in PCR in terms of purity and concentration. In addition, the template needs to 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 the Bioline ISOLATE II RNA Mini Kit (BIO-52072).

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

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.

Optional Fluorescein well-factor correction: SYBR® Fluorescein Kit is premixed with fluorescein, so that fluorescence emitted by fluorescein can be optionally detected on certain real-time instruments. If your real-time instrument has the capability of using fluorescein and you wish to use this option, then this option must be selected by the user in the software.

Troubleshooting Guide:

See the Bioline full documentation for detailed troubleshooting instructions.

http://www.bioline.com/us/downloads/dl/file/id/2667/sensifast_sybr_fluorescein_kit_manual.pdf

Materials

SensiFAST™ SYBR® & Fluorescein Kit BIO-96002 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 SYBR® & Fluorescein Mix	10 µL	1x
10 µM Forward Primer	0.8 µL	400 nM
10 µM Reverse Primer	0.8 µL	400 nM
Template	up to 8.4 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 real-time PCR conditions:

Step 3.

The following real- time PCR conditions are suitable for the SensiFAST SYBR® Fluorescein Kit with the amplicons of up to 200 bp. However, the cycling conditions can be varied to suit different machine-specific protocols. It is not recommended to use annealing temperatures below 60°C or combined annealing/extension times longer than 30 seconds.

Suggested real-time PCR conditions:

Step 4.

SensiFAST SYBR® Fluorescein Kit is compatible with either three-step or two-step cycling:

- 3-step cycling

Cycles	Temp.	Time	Notes
1	*95°C	*2 min	Polymerase activation
40	95°C 60-65°C 72°C	5 s 10 s **5-20s	Denaturation Annealing Extension (acquire at end of step)

*2 min for cDNA, 3 min for genomic DNA **Not recommended to extend beyond 20 seconds

Suggested real-time PCR conditions:

Step 5.

SensiFAST SYBR® Fluorescein Kit is compatible with either three-step or two-step cycling:

- 2-step cycling

Cycles	Temp.	Time	Notes
1	*95°C	*2 min	Polymerase activation
40	95°C 60-65°C	5s **15-30 s	Denaturation Annealing/extension (acquire at end of step)

*2 min for cDNA, 3 min for genomic DNA **Not recommended to anneal/extend beyond 30 seconds

Optional analysis

Step 6.

After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis.