SensiFAST™ HRM Kit

Bioline

Abstract

The SensiFAST™ HRM Kit has been developed for fast, highly reproducible High Resolution Melt (HRM) analysis and has been validated on commonly used real-time instruments. A combination of the latest advances in buffer chemistry and enhancers, together with an antibody-mediated hot-start DNA polymerase system, ensures that the SensiFAST HRM Kit delivers fast, highly-specific and ultra-sensitive HRM analysis.

For ease-of-use and added convenience, SensiFAST HRM is provided as a 2x mastermix containing all the components necessary for real-time PCR, including the EvaGreen® dye, dNTPs, stabilisers and enhancers. As a ready-to-use premix, only primers and template need to be added.

Citation: Bioline SensiFAST™ HRM Kit. **protocols.io**
dx.doi.org/10.17504/protocols.io.fyrbpv6
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Guidelines

Kit components

Reagent	200 x 20 µL reactions	500 x 20 µL reactions	2000 x 20 µL reactions
SensiFAST HRM mix (2x)	2 x 1 mL	5 x 1 mL	20 x 1 mL

General considerations

To help prevent any carry-over DNA contamination, we recommend that separate areas be maintained for PCR set-up, PCR amplification and any post-PCR gel analysis. It is essential that any amplified PCR product should not be opened in the PCR set-up area.

Primers: The sequence and concentration of the 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 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 400 nM is suitable for most reactions, however to determine the optimal concentration we recommend titrating in the range 0.1-1 µM
- use an equimolar primer concentration

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 following should be considered when using genomic DNA 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 Mini Kit (BIO-52066) for high yield and purity from both prokaryotic and eukaryotic sources

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3 mM, which is optimal for SensiFAST HRM in the majority of real-time PCR conditions. If necessary, we suggest titrating $MgCl_2$ 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), replacing the template with PCR-grade water.

Troubleshooting Guide

See the Bioline full documentation for detailed troubleshooting instructions.

http://www.bioline.com/us/downloads/dl/file/id/2705/sensifast_hrm_kit_manual.pdf

Materials

SensiFAST™ HRM Kit BIO-32002 by Bioline

Protocol

Reaction mix composition

Step 1.

Prepare a PCR master mix. 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 HRM Mix	10 µL	1x
10 µM Forward Primer	0.8 µL	400 nM
10 µM Reverse Primer	0.8 µL	400 nM
H_2O	up to 16 µL	
Template	4 µL	
	20 µL Final Volume	

Suggested thermal cycling conditions

Step 2.

SensiFAST HRM Kit is compatible with either 3-step or 2-step cycling:

- 3 step cycle

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

*2min for cDNA, 3min for genomic DNA

** Not recommended to extend beyond 20 seconds

- 2-step cycle

Cycles	Temperature	Time	Notes

1	*95°C	*2 min	Polymerase activation
40	95°C	5 s	Denaturation
	60-65°C	**15-30 s	Annealing/extension (aquire at end of step)

*2min for cDNA, 3min for genomic DNA

** Not recommended to extend beyond 20 seconds

ANNOTATIONS

Lenny Teytelman 14 Oct 2016

The above real-time PCR conditions are suitable for the SensiFAST HRM Kit with the amplicons of up to 200bp. 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.

HRM analysis

Step 3.

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