Associated Products

Product	Description	Pack Size	Cat No.
ISOLATE II Genomic DNA kit	Rapid isolation of DNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52065 BIO-52066 BIO-52067
ISOLATE II Plant DNA kit	Rapid isolation of DNA from a variety of plant samples	10 Preps 50 Preps 250 Preps	BIO-52068 BIO-52069 BIO-52070
ISOLATE II RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52071 BIO-52072 BIO-52073
ISOLATE II Plant RNA Kit	Fast and efficient isolation of extremely pure total RNA from a variety of plant samples	10 Preps 50 Preps	BIO-52076 BIO-52077
TRIsure™	Quick isolation of high-quality RNA from a variety of sources for subsequent use in cDNA synthesis	100 mL 200 mL	BIO-38032 BIO-38033
Tetro cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length cDNA from RNA	30 Reactions 100 Reactions	BIO-65042 BIO-65043
Agarose	Molecular biology grade agarose	100 g 500 g	BIO-41026 BIO-41025

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.

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Storage and Stability:
SensiFAST HRM Kit is shipped on dry/blue ice. All kit components should be stored at -20 °C upon receipt. Excessive freeze/thawing is not recommended. Thaw, mix, and briefly centrifuge each component before use.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Quality Control:
SensiFAST HRM 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:
Please refer to the material safety data sheet for further information.

Notes:
For research or further manufacturing use only.

SensiFAST™ HRM Kit

Shipping: On Dry/Blue Ice
Batch No.: See vial
Concentration: See vial

Catalog Numbers
BIO-32005: 500 x 20 µL reactions: 5 x 1 mL
BIO-32020: 2000 x 20 µL reactions: 20 x 1 mL

Store at –20°C

meridian BIOSCIENCE™

Description

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 master mix containing all the components necessary for real-time PCR (qPCR), including the EvaGreen® dye, dNTPs, stabilisers and enhancers. As a ready-to-use premix, only primers and template need to be added.

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 qPCR. We strongly recommend taking the following points into consideration when designing and running your qPCR:

- 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 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 qPCR 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.

Procedure

Reaction mix composition: 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	

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Suggested thermal cycling conditions

The following q 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.

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

- **3-step cycle**

***2 min for cDNA, 3 min for genomic DNA**

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 (acquire at end of step)

**** Not recommended to extend beyond 20 seconds**

- **2-step cycle**

Cycles	Temperature	Time	Notes
1	*95 °C	*2 min	Polymerase activation
40	95 °C 60-65 °C	5 s **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**

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

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	For complex templates such as genomic DNA increase inactivation time up to a minimum of 3 minutes.
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer design software or validated primers. Test primers on a control template
	Incorrect concentration of primers	Use primer concentration between 100 nM and 1 μM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
	Primers degraded	Use newly synthesized primers
	Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR-grade water
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling

Troubleshooting Guide (Continued)

Problem	Possible Cause	Recommendation
Non-specific amplification product AND Primer-dimers	Suboptimal primer design	Redesign primers using appropriate software or use validated primers
	Primer concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primer concentration too low	Titrate primers in the concentration range of 100 nM - 1 μM
	Primer annealing/extension temperature(s) too low	Due to the high ionic strength of SensiFAST HRM Kit it is not recommended to use annealing/extension temperatures below 60 °C. Annealing/extension temperature can be increased in steps of 2 °C in the event of non-specific products
	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
Variability between replicates	Error in reaction set-up	Prepare large volume master mix
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument
Late amplification trace	Activation time too short	Ensure the reaction is activated for between 1 min and 3 min at 95 °C before cycling
	Extension time too short	Increasing the extension time may be necessary for amplification products over 200 bp, double extension time to determine whether the cycle threshold (CT) is affected
	Annealing temperature too high	Decrease annealing temperature in steps of 2 °C
	Template concentration too low	Increase concentration if possible
	Template with high secondary structure	Increase reverse transcription reaction time up to 30 min 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	Redesign primers using appropriate software or use validated primers
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer concentration too low	Increase concentration of primer in 100 nM increments
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
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 water
	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