SensiMix™ HRM Kit

Shipping: On Dry/Blue Ice

Exp. Date: See vial

Batch No.: See vial

Concentration: see vial

Catalog Numbers

QT805-02: 250 x 25µl reactions: 5 x 625µl

QT805-05: 500 x 25µl reactions: 10 x 625µl

QT805-20: 2000 x 25µl reactions: 40 x 625µl

BIOLINE

Store at –20°C

Storage and Stability:
Store SensiMix HRM kit at -20°C. Avoid exposure of the EvaGreen solution to light.
Do not store the mix with EvaGreen in it. Always prepare the mix fresh, prior to a reaction.
When stored under these conditions, the reagents are stable for a minimum of 6 months from date of purchase

Quality Control:
Bioline operates under ISO 9001 Management System. The SensiMix HRM Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination, absence of nucleic acid contamination and to minimize batch-to-batch variation prior to release.

Safety Precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Description

High Resolution Melt (HRM™) analysis is the evaluation of nucleic acid sequences by their dissociation profiles. An amplification of the template is carried out in the presence of EvaGreen™ dye and the subsequent products are subjected to a melt step. The melt behavior of a sequence will depend on its composition and length and therefore even sequences differing by one base will have slightly different melt profiles. Until recently, the ability to detect such sequences has been down to using separate probes which bind over the area containing the mutation. However the advent of fast melting machines has made detection of single nucleotide polymorphisms (SNPs) possible, using DNA binding dyes.

SensiMix™ HRM is a 2x mix which has been optimized for HRM analysis on the Corbett Life Science Rotor-Gene™ 6000 HRM system. It is designed for use with the EvaGreen dye supplied.

Kit components

Reagent	Pack Size			Description
	250 x 25 µl reactions	500 x 25 µl reactions	2000 x 25 µl reactions	
SensiMix HRM™	5 x 625µl	10 x 625µl	40 x 625µl	2x HRM mix containing reaction buffer, heat-activated DNA polymerase, dNTPs, 6mM $MgCl_2$ and stabilizers
EvaGreen™	1 x 250µl	2 x 250µl	8 x 250µl	Fluorescent intercalating dye
50mM $MgCl_2$	1 x 1ml	1 x 1ml	2 x 1ml	Molecular biology grade $MgCl_2$ for optimization of SensiMix HRM

Evaluation of SensiMix HRM Sample

If you have received this mix as a sample 15-reaction size, we recommend performing HRM on multiple replicates of each sample.

Reaction conditions / suggestions for use

The preparation of a reaction master mix is recommended:

- It is advised in general to make up at least one extra volume of reaction master mix than for the number of samples required (e.g. Make up a master mix sufficient for 9 samples if 8 are required).

- The inclusion of no template control (NTC) samples is also recommended, i.e. add dH_2O in place of a template.

Hot start polymerase
The polymerase in SensiMix HRM is inactive at room temperature and a 10-minute hot start at 95°C is essential to activate the enzyme.

Primer concentration
In most cases a final primer concentration of 250nM is sufficient. However, primer concentration can be optimized within the 25 - 900nM range.

Template concentration
An excess of template will inhibit reactions. 100ng of human cDNA in a 25µl reaction is normally sufficient. However this may vary, depending on the particular gene of interest.

$MgCl_2$ Optimization
$MgCl_2$ is a co-factor for the DNA polymerase. A final $MgCl_2$ concentration of 3mM should be ideal for most reactions. However, the $MgCl_2$ requirements for the polymerase often vary, depending on the particular template and primers used. If it is necessary to alter the $MgCl_2$ concentration, we would recommend performing a series of 25µl reactions in triplicate, with varying final concentrations of $MgCl_2$ on a dilution series of suitable templates.

Typical protocol for Corbett Life Science Rotor-Gene 6000

Components	Volume (µl)	Final Concentration
SensiMix HRM	12.5	1x
EvaGreen dye	1	-
Primer mix (5 µM)	1	200nM
DNA template	5	determined by customer
$MgCl_2$	*	determined by customer
dH_2O	Up to 25µl	
Total volume	25µl	

Extension conditions:

Cycles	Temperature	Time	Notes
1	95°C	*10min	Polymerase activation
40	95°C X°C 72°C	15s 10s 10s	dependent on Tm of primers
Acquire on GREEN channel			

High Resolution Melt conditions:

Ramp from 75°C to 90°C, rising by 0.1°C each cycle
Acquire on HRM channel

Optional: Check the specificity of PCR products by running the products on a Tris-Acetate-EDTA agarose gel.

Considerations when designing your HRM assay

1. Amplicon size: We would recommend keeping the amplicon size between 100 and 200 base pairs, with the mutation located towards the middle. Amplicons of up to 250 base pairs can be used, although significant optimization may be required. However, there may be additional problems with longer fragment lengths, for example, multiple melt domains. With small amplicons, the effect of a mutation is greater on the overall melt temperature of the fragment. However, it is advisable to avoid amplicon sizes of less than 100 bases if possible.

2. Primer Design: Perform a thorough analysis of the region eg using Ensembl (http://www.ensembl.org), BLAST (http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html) etc, looking for intron/exon boundaries, variations in the template, secondary structure etc. This will ascertain good regions within which your primers can be designed.

3. Multiple primer screening: Design at least 3 forward and 3 reverse primers e.g. using primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), with melting temperatures of around 60°C.

4. Optimize: Run all possible combinations of forward and reverse primers and analyze the normalized melt data and difference plots for each pairing. Select the primer pair which gives the most distinctive curves (see figure 1). It is advisable that the best primer pair is also run on an agarose gel to check specificity.

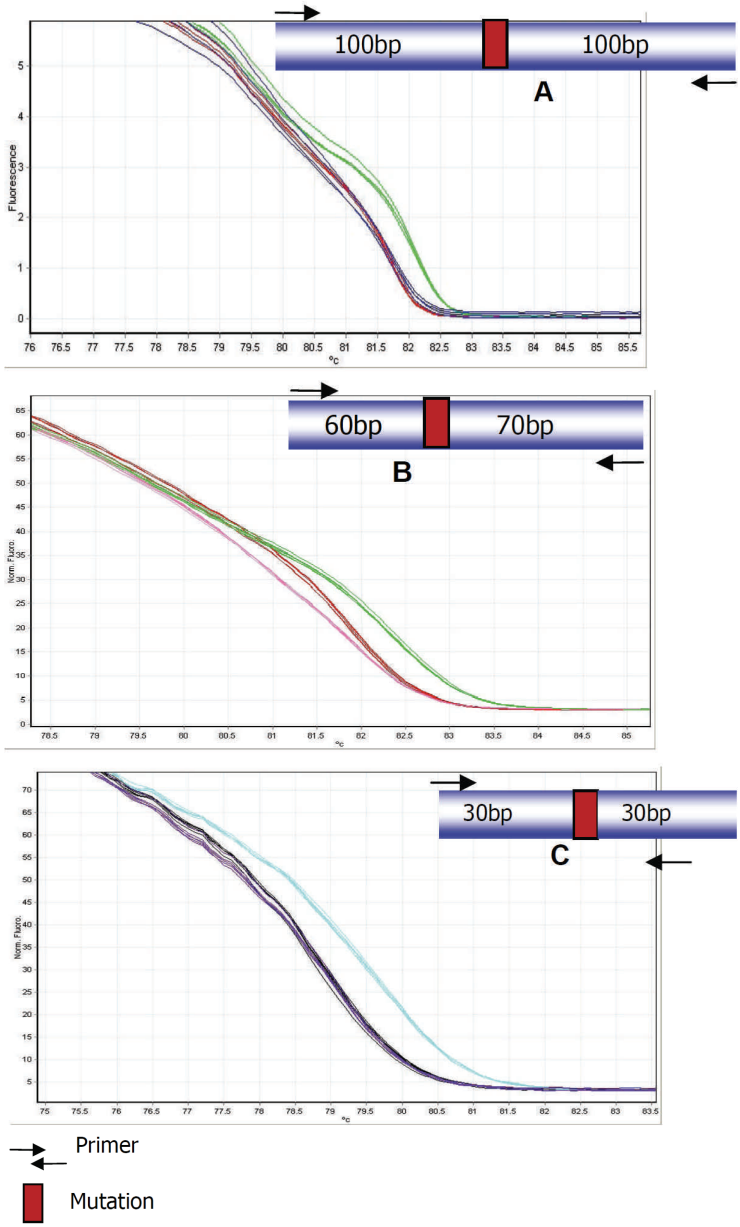

Figure 1: Three separate HRM runs using identical DNA samples containing homoduplex wild-type, homoduplex mutant and a heteroduplex, using 3 different primer pairs. This figure illustrates the advantage of screening multiple primer pairs, with the middle melt curve showing clearer separation between the genotypes.

Notes on licences:

1. Products and procedures described in this protocol are intended for research purposes only
2. SensiMix™ is a trademark of Bioline Reagents Ltd
3. Rotor-Gene™ and HRM™ are trademarks of Corbett Life Science
4. Purchase of this product includes limited rights to use EvaGreen™ Stain patented by Biotium Inc. EvaGreen™ is a trademark of Biotium Inc.
5. Purchase of this product does not convey a licence to perform any patented process

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