



SensiFAST™ HRM Kit

Precision Perfected

- **Accurate:** clear discrimination of even the most challenging sequence differences
- **Reproducible:** unparalleled consistency between replicate difference plots for increased confidence in sample characterization
- **Sensitive:** efficient detection from even very limiting amounts of sample
- **Flexible:** enables reliable characterization of a broad range of sequence differences
- **Fast:** rapid PCR amplification prior to high resolution melting, enabling higher throughput

SensiFAST™ HRM Kit incorporates an antibody-mediated hot-start DNA polymerase with EvaGreen® fluorescent dye, to deliver precise, highly reproducible and fast genotyping of sequence variants through high resolution melting.

SensiFAST HRM Kit has been developed for detailed characterization of samples according to their base composition, length and GC content by high resolution melting (HRM). The latest advances in buffer chemistry and enhancers, together with an antibody-mediated hot-start DNA polymerase, ensure SensiFAST HRM Kit delivers reproducible, accurate HRM analysis. SensiFAST HRM Kit enables reliable detection of even single base changes, making it suitable for genotyping single nucleotide polymorphisms (SNPs) (Table 1).

Table 1. Classes of SNP Genotyping

SNP Classes	Base Change	T _m - Curve Shift	Frequency in Humans
1	C/T and G/A	Large > 0.5 °C	65 %
2	C/A and G/T	Large > 0.5 °C	19 %
3	C/G	Small > 0.2-0.5 °C	9 %
4	A/T	Small > 0.2 °C	7 %

APPLICATIONS

- SNP genotyping
- Characterization of haplotype blocks
- DNA mapping
- Detection of insertions, deletions and translocations
- DNA fingerprinting
- DNA methylation analysis
- Gene scanning
- Species identification
- Allelic prevalence population studies
- Screening for loss of heterozygosity

PRECISE, HIGHLY REPRODUCIBLE GENOTYPING

SensiFAST HRM Kit contains EvaGreen, a third generation saturating fluorescent dye which selectively binds to double-stranded DNA. In contrast to dyes such as SYBR® Green I, EvaGreen can be used at higher concentrations without inhibiting PCR and shows equal binding affinity for GC-rich and AT-rich regions. The combination of SensiFAST DNA Polymerase, a unique buffer system and EvaGreen dye enables amplification and discrimination of even the most challenging sequence differences, such as class 4 SNPs (Fig. 1), without sequence preference (Fig. 2). Since it does not require expensive labelled oligonucleotide probes, SensiFAST HRM Kit is also a cost effective alternative to traditional probe based genotyping methods.

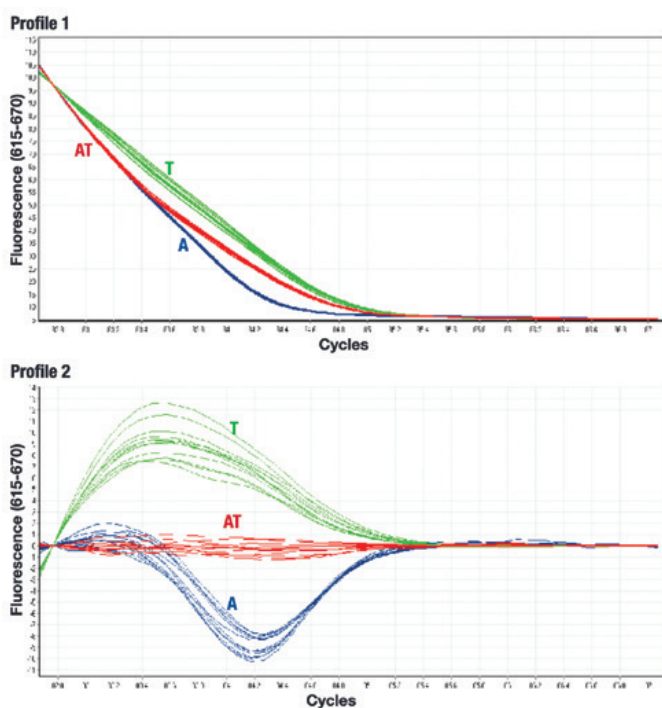


Fig. 1 Highly accurate genotyping of an A/T Class 4 SNP

Three different genotypes of an A>T SNP were analyzed by HRM, using SensiFAST HRM Kit. Normalized HRM melt profiles of each genotype are shown (Profile 1), where A (blue) corresponds to the homozygous wild type, T (green) to the homozygous mutant and A/T (red) to heterozygous samples. A difference plot of this data (Profile 2) shows the effect of subtracting an average heterozygous curve from all curves. The results illustrate clear discrimination and highly accurate results even with class 4 SNPs when using SensiFAST HRM.

	Hue	Name	Genotype	Confidence %
1	Blue	SF HRM Monozygote A	A	99.04
2	Blue	SF HRM Monoduplex A	A	99.51
3	Blue	SF HRM Monoduplex A	A	99.53
4	Blue	SF HRM Monoduplex A	A	99.66
5	Blue	SF HRM Monoduplex A	A	99.15
6	Blue	SF HRM Monoduplex A	A	98.02
7	Blue	SF HRM Monozygote A	A	98.24
8	Blue	SF HRM Monoduplex A	A	98.41
9	Blue	SF HRM Monoduplex A	A	99.64
10	Blue	SF HRM Monoduplex A	A	99.83
11	Blue	SF HRM Monoduplex A	A	99.48
12	Blue	SF HRM Monoduplex A	A	98.99
13	Green	SF HRM Monoduplex T	T	99.36
14	Green	SF HRM Monoduplex T	T	99.71
15	Green	SF HRM Monoduplex T	T	99.85
16	Green	SF HRM Monoduplex T	T	99.2
17	Green	SF HRM Monoduplex T	T	99.87
18	Green	SF HRM Monoduplex T	T	99.13
19	Green	SF HRM Monoduplex T	T	99.46
20	Green	SF HRM Monoduplex T	T	99.72
21	Green	SF HRM Monoduplex T	T	99.44
22	Green	SF HRM Monoduplex T	T	99.01
23	Green	SF HRM Monoduplex T	T	99.77
24	Green	SF HRM Monoduplex T	T	99.83
25	Red	SF HRM Hetroduplex AT	AT	99.24
26	Red	SF HRM Hetroduplex AT	AT	99.73
27	Red	SF HRM Hetroduplex AT	AT	99.66
28	Red	SF HRM Hetroduplex AT	AT	99.89
29	Red	SF HRM Hetroduplex AT	AT	99.69
30	Red	SF HRM Hetroduplex AT	AT	99.94
31	Red	SF HRM Hetroduplex AT	AT	99.36
32	Red	SF HRM Hetroduplex AT	AT	99.85
33	Red	SF HRM Hetroduplex AT	AT	99.39
34	Red	SF HRM Hetroduplex AT	AT	99.89
35	Red	SF HRM Hetroduplex AT	AT	99.79
36	Red	SF HRM Hetroduplex AT	AT	99.85

Fig. 2 Auto-calling of genotypes

Each sample from figure 1 was automatically genotyped using standard genotyping software, the results illustrate clear discrimination of SNPs using SensiFAST HRM, with all samples achieving over 98% confidence level.

Ordering Information

SensiFAST™ HRM Kit	Size	Cat. #
SensiFAST HRM Kit	500 Reactions	BIO-32005
	2000 Reactions	BIO-32020

For related products such as RNA isolation kits, and qPCR kits visit www.bioline.com

Contact information:

Global

E: info@meridianlifescience.com
Toll free: +1 800 327 6299

Australia

E: info.au@meridianlifescience.com
Tel: +61 (0)2 9209 4180

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