AccuMelt HRM SuperMix

Maximize differences in melting temperature to discriminate DNA sequences between samples

FEATURES AND BENEFITS:

- See subtle sequence differences clearly – robust amplification ensures sufficient yield of products to generate discrete melt curves
- Accurate genotype calling – comparable or better performance than TaqMan® genotyping
- Work with rare or precious samples – large range of template inputs possible
- Specificity – works with lower Mg^{2+} concentration than other systems thus enhancing assay accuracy

DESCRIPTION:

AccuMelt HRM SuperMix is a ready-to-use 2x concentrated hot-start PCR mix containing SYTO® 9 green fluorescent DNA-binding dye.

Superior Resolution of Genotypes

SNP genotyping is a useful application for HRM and illustrates the capabilities of AccuMelt HRM SuperMix. Genotypes are readily identified based on unique melting profiles depending on a sample's sequence (Figure 1). Furthermore, AccuMelt HRM SuperMix gives superior resolution of difficult genotypes when compared to the leading competitor's mix based on greater T_m differences observed for A → T transversions (Figure 2).
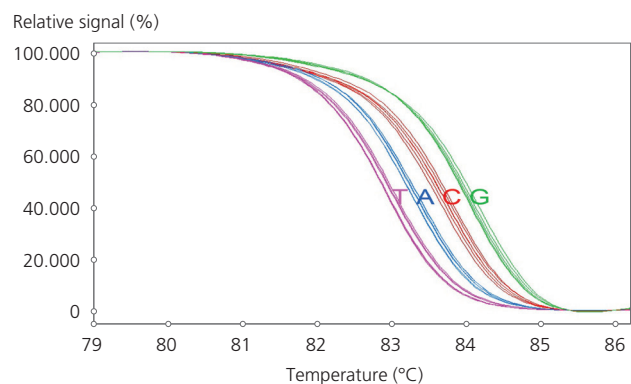

Figure 1 High resolution melting analysis of a model SNP system with a single A, C, G, or T variant base. AccuMelt HRM SuperMix readily resolves each genotype and T_m differences are easily visualized in normalized melting curve plots (Roche Lightcycler 480).
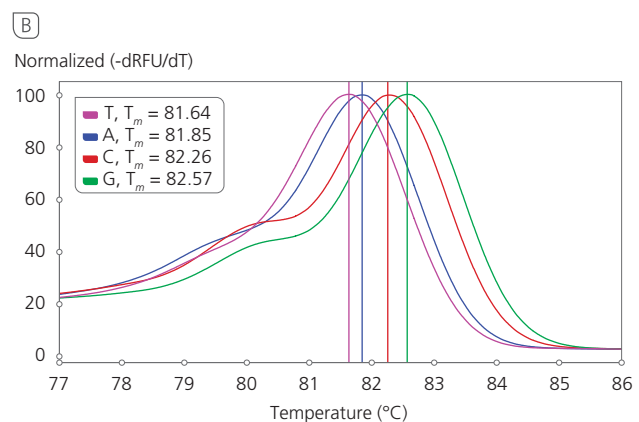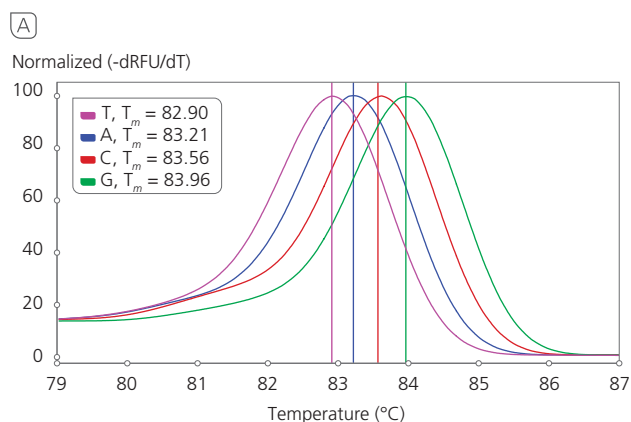

Figure 2 Effect of T, A, C or G variant base on T_m in a model HRM SNP system with either AccuMelt HRM SuperMix (Panel A), or a competitor's SYTO 9 dye master mix (Panel B). Plots of averaged melt peaks normalized to maximum signal for each system.

Comparison to TaqMan Genotyping

TaqMan genotyping has been used successfully in SNP analysis and other allelic discrimination applications. This widely adopted standard in genotyping was used as a benchmark to assess the utility of HRM with our SuperMix. AccuMelt HRM was determined to be just as effective as TaqMan genotyping in SNP analysis and was even able to call the genotype for a difficult sample which the TaqMan assay could not resolve (Figure 3).

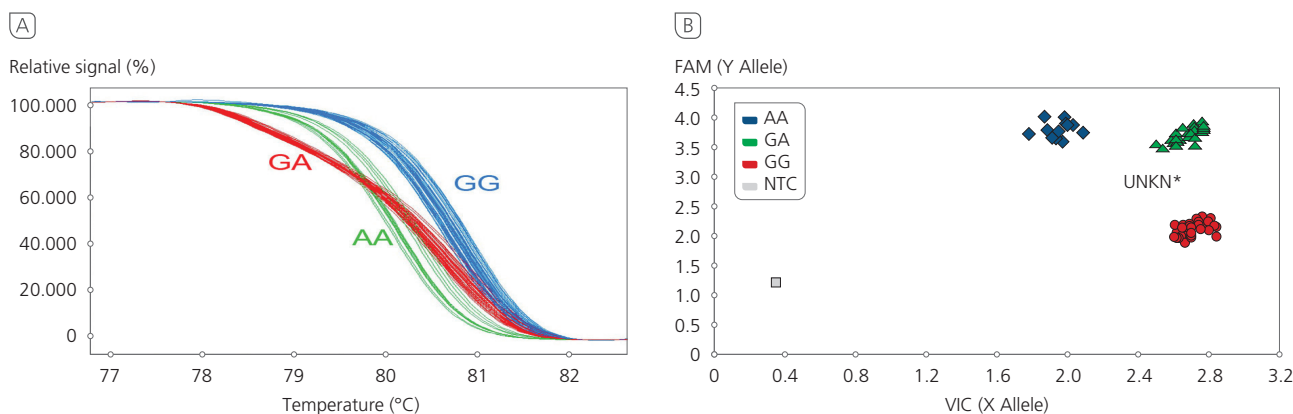### Comparison to TaqMan Genotyping

Figure 3 Accuracy of HRM genotyping with AccuMelt HRM SuperMix was evaluated by comparison to TaqMan detection of the G>A rs1801133 SNP in the MTHFR gene (Panel A). HRM normalized melting curves (Panel B). TaqMan allelic discrimination plots. TaqMan failed to resolve Sample D3 (labeled as "UNKN") which was typed as a heterozygote by HRM.

Robust Amplification

Consistent robust amplification is critical to accuracy in HRM analysis. AccuMelt HRM SuperMix will drive all PCR amplifications to plateau regardless of the quantity of template input (Figure 4). This ensures accurate results regardless of the quantity of DNA available.

Figure 4 High yield, high efficiency PCR with AccuMelt HRM SuperMix. Real-time PCR of GAPDH was amplified from log-fold serial dilutions of qScript synthesized cDNA from HeLa cell total RNA (10 ng to 0.1 pg) was carried out with either a leading SYBR Green Master Mix or AccuMelt HRM SuperMix using the following cycling conditions: 95°C, 20 s; followed by 45 cycles of: 95°C, 3 s; 60°C, 20 s. Averaged plots for quadruplicate reactions for each input quantity are shown.
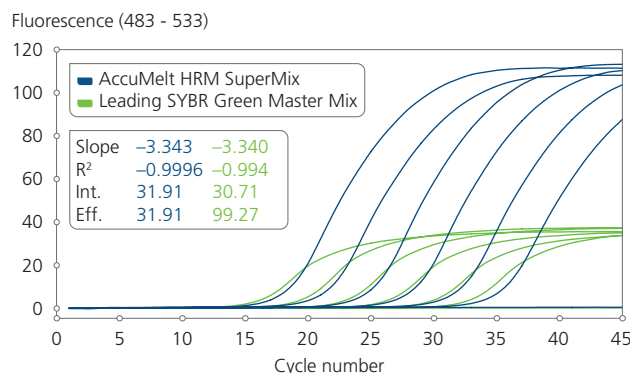
ORDER INFO

Product Name	Quantabio Catalog Number	Size
AccuMelt HRM SuperMix - 250 R	95103-250	250 x 20 µl rxns (2 x 1.25 ml)
AccuMelt HRM SuperMix - 1250 R	95103-012	1250 x 20 µl rxns (10 x 1.25 ml)

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