

AccuMelt™ HRM SuperMix

Cat. No.	95103-250	Size:	250 x 20- μ L reactions (2 x 1.25 mL)
	95103-012		1250 x 20- μ L reactions (10 x 1.25 mL)
	95103-05K		5000 x 20- μ L reactions (2 x 25 mL)

Store at **-20°C** protected from light

Description

AccuMelt HRM SuperMix is a 2X concentrated, ready-to-use reaction cocktail for detection of genetic variations using high resolution melting (HRM) analysis. It includes all required components except for primers and DNA template. HRM is a closed tube, rapid and cost effective procedure for characterization of sequence differences immediately following PCR amplification. It is based on the melting (dissociation) behavior of a PCR product as it transitions from double-stranded to single-stranded DNA in the presence of a fluorescent dsDNA-binding dye. The melting properties of a given PCR product are dependent upon the base composition, length, and strand base-pairing. HRM analysis tools exploit differences in melt curve shapes and DNA melting temperature (T_m) to discriminate sequence differences between samples.

AccuMelt HRM SuperMix contains the green-fluorescent dye SYTO® 9 in a stabilized master mix that eliminates the need for time consuming optimization of critical PCR components. This dye provides strong fluorescent signal upon binding to dsDNA at saturating concentrations without inhibiting PCR. The unique chemical composition of this SuperMix further enhances and maximizes the impact of sequence variations on melt curve behavior. This facilitates discrimination of all sequence variations including difficult to resolve base-neutral transversions such as A>T class 4 SNPs. The dNTP mix in AccuMelt HRM SuperMix includes an optimized blend of dTTP and dUTP. This feature supports the optional use of uracil-DNA glycosylase (UNG) to prevent amplification of carry-over contamination, while providing high product yield and reliable PCR performance.

Highly specific amplification with high product yield from complex genomic DNA template is critical for successful HRM studies. A key component of this SuperMix is AccuStart™ Taq DNA polymerase, which contains monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation at 95°C, the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly. AccuMelt HRM SuperMix can be used with all currently available HRM analysis systems. For HRM applications and more detailed product information, please visit our web site at www.quantabio.com.

Components

AccuMelt HRM SuperMix (2X):	2X reaction buffer containing optimized concentrations of MgCl ₂ , dNTPs (including dUTP), AccuStart Taq DNA Polymerase, SYTO 9 green-fluorescent dye, and stabilizers. [free Mg ⁺⁺] = 0.8 mM at 1X final concentration
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Storage and Stability

AccuMelt HRM SuperMix is stable for 1 year when stored in a constant temperature freezer at -20°C, protected from light. For convenience, it may be stored unfrozen at +2 to +8°C for up to 6 months. After thawing, mix thoroughly by gently vortexing tube contents. Centrifuge briefly to collect contents before using.

While repeated freezing and thawing of the product is not recommended; AccuMelt HRM SuperMix demonstrated no loss of performance after 20 cycles of freezing and thawing.

Guidelines for PCR amplification and HRM analysis:

- The design of highly specific primers is the single most important parameter for successful PCR amplification and HRM analysis. The use of computer aided primer design programs is encouraged in order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer and the primer pair. Primer T_m should be between 56 to 63°C and the T_m difference between the forward and reverse primers should be less than 2°C.
- Amplicon size should be less than 250 bp. Smaller amplicons (60 to 100 bp) generally facilitate HRM discrimination of homozygote samples. We recommend designing and evaluating multiple primer set designs for any given application.
- Optimal primer concentration may vary between 100 and 500 nM. A final concentration of 300 nM for each primer is effective for most applications. Some primer set designs may require asymmetric primer concentrations.
- Always include a negative or no template control to evaluate the specificity of a given primer set and amplification protocol. Sequence specificity of the PCR should be confirmed by a method other than generation of a single melt peak. This includes confirmation of PCR product size, diagnostic restriction endonuclease fragment pattern, or sequencing.
- Preparation of a reaction cocktail is recommended to reduce pipetting errors and obtain reproducible HRM results. Assemble the reaction cocktail with all required components, except template DNA, and dispense equal aliquots into each reaction tube. Add DNA template as a final step. A minimum of 3 technical replicates for each DNA sample is recommended. Include appropriate positive controls for each sequence variant.

Guidelines for PCR amplification and HRM analysis continued:

- Use approximately 10,000 copies of template DNA. A suggested input quantity for human genomic DNA is 10 to 30 ng.
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.
- PCR amplification can be carried out in a conventional or real-time thermal cycler. Monitoring the reaction in real-time allows one to access the quality and PCR performance of a sample before HRM. All samples should produce comparable C_qs and fluorescence signal. Samples with delayed C_q (>30) or aberrant fluorescence signal should be excluded from the HRM analysis. Optimal cycling conditions will depend on the properties of your primers. Hold assembled reactions on ice, protected from light, if not proceeding immediately to PCR.

Reaction Assembly

Component	Volume for 20- μ L rxn.	Final Concentration
AccuMelt HRM SuperMix (2X)	10.0 μ L	1x
Forward primer	Variable	100 – 500 nM
Reverse primer	Variable	100 – 500 nM
Nuclease-free water	Variable	
DNA Template	2-5 μ L	~10,000 copies
Final Volume (μ L)	20 μ L	

PCR Cycling Protocol

	2-Step Cycling Protocol	3-Step Cycling Protocol
Initial Denaturation	95°C, 5 min*	95°C, 5 min*
PCR cycling (40 to 45 cycles)		
Denaturation	95°C, 5 to 10 s	95°C, 5 to 10 s
Annealing	60°C, 30 s [†]	15 s at 55 to 65°C
Extension		10 to 30s at 70°C [†]
HRM analysis [§]	Consult instructions for your instrument	

* Full activation of AccuStart Taq DNA polymerase occurs within 30s at 95°C; however, optimal initial denaturation time is *template dependent* and will affect PCR efficiency and sensitivity. Amplification of genomic DNA or supercoiled DNA targets may require 5 to 10 min at 95°C to fully denature the template.

[†] If monitoring PCR in real-time, collect and analyze kinetic PCR data at the end of the extension step. Extension time is dependent upon amplicon length and minimal data collection time requirement for your qPCR instrument. Some primer sets may require a 3-step cycling protocol for optimal performance. Optimal annealing temperature and time may need to be empirically determined for any given primer set.

[§] **High Resolution Melting Analysis** should be carried out immediately following PCR amplification. *Please consult the instructions for your HRM instrument for procedural details.* If not proceeding immediately to HRM, store plates at +4C protected from light. Mix and centrifuge samples immediately before HRM.

Quality Control

Kit components are free of contaminating DNase and RNase. AccuMelt HRM SuperMix is functionally tested to amplify a single copy gene in human genomic DNA and resolve homozygote samples for class 4 (A>T) and class 3 (C>G) SNPs in a model test system.

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