

## AccuMelt™ HRM SuperMix

Cat. No. 95103-250      Size: 250 x 20- $\mu$ L reactions (2 x 1.25 mL)  
95103-012                      1250 x 20- $\mu$ L reactions (10 x 1.25 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](http://www.quantabio.com).

### Components

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

### 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 Cqs and fluorescence signal. Samples with delayed Cq (>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- $\mu$ L rxn. | Final Concentration |
|----------------------------|-----------------------------|---------------------|
| AccuMelt HRM SuperMix (2X) | 10.0 $\mu$ L                | 1x                  |
| Forward primer             | Variable                    | 100 – 500 nM        |
| Reverse primer             | Variable                    | 100 – 500 nM        |
| Nuclease-free water        | Variable                    |                     |
| DNA Template               | 2-5 $\mu$ L                 | ~10,000 copies      |
| Final Volume ( $\mu$ L)    | 20 $\mu$ 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.

### Limited Label Licenses

This product is provided under an agreement between Life Technologies Corporation (Molecular Probes Labeling and Detection Technologies) and Quanta BioSciences, Inc. and the manufacture, use, sale, or import of this product is subject to one or more U.S. Patents and corresponding international equivalents. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer, where such research does not include testing, analysis or screening services for any third party in return for compensation on a per test basis. The sale of this product is expressly conditioned on the buyer not using the product or its components (1) in manufacturing; 2) to provide a service, information, or data to an unaffiliated third party for payment; 3) for therapeutic, diagnostic or prophylactic purposes; 4) for resale, whether or not such product or its components are resold for use in research; or for any other commercial purpose. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation (Molecular Probes Labeling and Detection Technologies), Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300, FAX: (541) 335-0354

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,994,056 and 6,171,785. The purchase of this product includes a limited, nontransferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The purchase of this product includes a limited, non-transferable license for all fields other than human or veterinary in vitro diagnostics under U.S. Patent No. 5,871,908, owned by Evotec Biosystems GmbH and licensed to Roche Diagnostics GmbH, to use only the enclosed amount of product according to the specified protocols. No right is conveyed, expressly, by implication, or by estoppel, to use any instrument or system under any claim of U.S. Patent No. 5,871,908, other than for the amount of product contained herein.

Licensed to Quanta BioSciences, under U.S. Patent Nos. 5,338,671, 5,587,287, and foreign equivalents.

AccuMelt and AccuStart are trademarks of Quanta BioSciences, Inc. SYTO is a registered trademark of Life Technologies Corporation (Molecular Probes Labeling and Detection Technologies).