



Addendum to HRM Manual

Protocols and Tips for the Complete HRM Solution.

We are pleased to bring you Agilent's High Resolution Melt (HRM) Master Mix as well as a Complete HRM Solution.

We believe this product has the opportunity to be the first choice for core labs, primary investigators and clinical researchers who already use HRM.

This communication will highlight key areas in the manual where you can find information on how to run the software and provide update recommendations for running the assay on the AriaMx Real-Time PCR System.

- **Pre-protocol considerations:** details can be found on page 11 of the manual. For quick reference:
 - Recommended Primer design tool: Primer 3, version 0.4.0 at <http://bioinfo.ut.ee/primer3-0.4.0/>
 - Mandatory calibration plate can be ordered using Agilent part number: 5190-7702. Calibrate yearly.

- **Cycling condition recommendations:** details are found on page 12. For quick reference:
 - Hot start = 3 minutes @95°C
 - Amplification = 40 cycles
 - HRM = ramp between 65°C and 95°C with a resolution of 0.2°C and a soak time of 10 sec

- **Reaction preparation:** details are found on page 13 in table 6. For Quick Reference:
 - The final Concentration of ROX in the reaction will be 30nM (if Rox is needed)
 - The final volume for the reaction will be 20µL

- **Running the reaction:** The experiment name is: Allele

Discrimination – DNA Binding Dye Including High Resolution Melt type experiment that can be found both at the instrument webpage, and the HRM software on the user's PC

- **Viewing the Melt Curves in AriaMx software:** Details can be found in the manual on page 15-17. For quick reference:
 - Navigate to the graphical display screen and select the melt curve raw/derivative curve graph
 - Set Fluorescence term to "R" or "Rn"
 - Make sure normalization is turned on
 - Adjust the temperature range as needed
 - Verify that curves for different populations have distinguishable shapes.

WHERE TO FIND RESOURCES

Technical support:

Genomics Division- Bioreagents team

800-227-9770-3-4-3 tel.

858 777 5387 fax

Collaborations: Please contact Mary Napier, Senior Product Manager Genomics and Molecular Diagnostics, or Caroline Tsou if you have any questions regarding this product.

Ordering:

5190-7702 AriaMx HRM Calibration Kit

5190-7827 Brilliant HRM Ultra-Fast Loci Master Mix (1 × 2mL)

5190-7909 Brilliant HRM Ultra-Fast Loci Master Mix Sample (15 reactions, 150µL)

Software updates: www.agilent.com/genomics/ariamxsoftware



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