MeltDoctor™ HRM Master Mix

Insert PN 4426225 Rev. B
Printed in USA

For Research Use Only.
Not for use in diagnostic procedures.

Product list

Part Number	Part	Storage Conditions
4415440	MeltDoctor™ HRM Master Mix, 5-mL bottle	On receipt, store at –15 to –25 °C for maximum stability. After the first use, the product may be stored at 2 to 8 °C for up to 3 months.
4415452	MeltDoctor™ HRM Master Mix, 5 × 5-mL bottle	
4415450	MeltDoctor™ HRM Master Mix, 10 × 5-mL bottle	
4409535	MeltDoctor™ HRM Master Mix, 50-mL bottle	

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Applied Biosystems High Resolution Melting Getting Started Guide* (PN 4393102). For all chemicals in **bold** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Perform an HRM calibration before using the MeltDoctor™ HRM Master Mix on the Applied Biosystems Real-Time PCR System.

For detailed procedures, refer to the *Applied Biosystems High Resolution Melting Getting Started Guide* (PN 4393102).

Reagent characteristics

The **MeltDoctor™ HRM Master Mix** contains magnesium chloride, dNTPs, **MeltDoctor™ HRM Dye**, and **AmpliTaq Gold® 360 DNA Polymerase** at a 2× concentration. The **MeltDoctor™ HRM Master Mix** is compatible with UDG degradation. Use the **MeltDoctor™ HRM Master Mix** with your primers to amplify your DNA for high resolution melting analysis.

Prepare the reactions

1. Add the required volumes of each component to an appropriately sized tube:

Components	Volume for one 20-µL reaction	Final concentration	Acceptable concentration range
MeltDoctor™ HRM Master Mix	10 µL	1×	–
Primer 1 (5 µM)	1.2 µL	0.3 µM	0.2 to 0.5 µM
Primer 2 (5 µM)	1.2 µL	0.3 µM	0.2 to 0.5 µM
Genomic DNA (20 ng/µL)	1.0 µL	1 ng/µL	10 pg/µL to 10 ng/µL
Deionized water	6.6 µL	—	–
Total volume	**20 µL**	**—**	

2. Cap the tube, then vortex to mix.
3. Spin the tube briefly.

Prepare the reaction plate

1. Pipet 20 µL of each reaction to the appropriate well of a reaction plate.
2. Seal the reaction plate with optical adhesive film, then spin the reaction plate.
3. Confirm that the liquid is at the bottom of the wells in the reaction plate.
4. Place the reaction plate on ice, protected from light, until you are ready to perform the run.

Run the reaction plate

1. Run the reaction plate using the recommended conditions:

Instrument and system software	Document/experiment properties	Thermal profile/run method settings
Applied Biosystems 7900HT Fast Real-Time PCR System, SDS Software v2.3	• Assay: Standard Curve (AQ) • Container: 96 Wells Clear Plate or 384 Wells Clear Plate • Template: Blank Template	• Passive Reference: None • Sample Volume (µL): 20 • Mode: Standard
Applied Biosystems 7500 Fast Real-Time PCR System, 7500 Software v2.0	• Instrument: 7500 Fast (96 Wells) • Experiment type: Quantitation-Standard Curve • Reagents: Other, select the Include Melt Curve checkbox • Ramp speed: Standard (~2 hours to complete a run)	• Reaction Volume Per Well: 20 µL • Passive reference: None • Expert mode: Select the Expert Mode checkbox • Filter selection: Select the Filter-1 checkbox

Note: If you are running a 96-well Fast reaction plate on the 7900HT system, perform the melt curve stage in a separate run. After the amplification run, remove the plate from the instrument, then spin the plate briefly before returning the plate to the instrument for the melt curve run.

Stage	Step	Temp	Time	Ramp rate (7900HT only)
Holding	Enzyme activation	95 °C	10 min	100%
Cycling (40 cycles)	Denature	95 °C	15 sec	100%
	Anneal/extend	60 °C	1 min	100%
Melt curve/dissociation	Denature	95 °C	10 sec	100%
	Anneal	60 °C	1 min	100%
	High resolution melting	95 °C	15 sec	1%
	Anneal	60 °C	15 sec	100%

2. Save the file.
3. Using the instrument system software, review the amplification results.
4. Using the Applied Biosystems High Resolution Melting Software, review the melt curves.

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