PCRBIOSYSTEMS
simplifying research

Clara™ HRM Mix

www.pcrbio.com

Product description:

Clara™ HRM Mix comprises our ultra-pure, Taq polymerase in a unique blend containing dNTPs and $MgCl_2$. The mix is powered by our third-generation DNA-intercalating SyGreen 2 dye for greatly reduced PCR inhibition. This new generation qPCR mastermix offers superior performance for accurate SNP discrimination and quantification of methylation differences.

HRM analysis is a powerful technique for the analysis of mutations, polymorphisms and epigenetic differences in double-stranded DNA samples.

Clara™ HRM Mix relies on our ultra-pure Taq polymerase, purified using our 12-step purification method to totally eliminate host DNA contamination and improve reaction sensitivity and specificity.

Our high-throughput smart-screen technology screening has resulted in a buffer system that allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions and results in higher senstitivity in distingushing every class of SNP.

Quality control

PCR Biosystems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

Pack Size	2x Clara™ HRM Mix
100 reactions	1 x 1 mL
500 reactions	5 x 1 mL
2000 reactions	20 x 1 mL

Shipping and storage

On arrival the kit should be stored between -30˚C and -15˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4˚C for 1 month.

Limitations of product use

This product is for research use only.

Technical support

Help and support is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions and our qPCR technical guide. For technical support and troubleshooting you can submit a technical enquiry online, or alternatively email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile

Instrument compatibility

Manufacturer	Instrument
Applied Biosystems	7900, 7900HT, 7900HT FAST, StepOne™, StepOne™ Plus, 7500, 7500 FAST, Viia7™
Bio-Rad®	CFX96™, CFX384™
Eppendorf	Mastercycler® ep realplex, Mastercycler® realplex 2S
Illumina®	Eco™
Qiagen/Corbett	6000, Q
Roche Applied Science	Lightcycler®480, Lightcycler® 96, Lightcycler®Nano
Bio Molecular Systems	Mic qPCR Cycler

Insturment Selection: Not all real time thermocyclers are suitable for HRM analysis. We provide a chart above for quick reference. You can also use our selection tool (https://pcrbio.com/resources/qpcr-selection-tool/). If your instrument is not listed please refer to the manufacturer's manual or get in touch with us at technical@pcrbio.com to find out whether or not it is suitable for HRM experiments.

Important considerations

Primer design: For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80bp and 200bp. With all manufacturers master mixes the shorter the amplicon length the faster the reaction can be cycled. Amplicon lengths should not exceed 400bp. Primers should have a predicted melting temperature of around 60˚C, using default Primer 3 settings (https://bioinfo.ut.ee/primer3/).

Reaction setup

1. Before starting, briefly vortex 2x Clara™ HRM Mix.
2. Prepare a master mix based on following table:

Reagent	20µL reaction	Final concentration	Notes
2x Clara™ HRM Mix	10 µL	1 x	
Forward primer (10 µM)	0.8 µL	400 nM	See above for optimal primer design
Reverse primer (10 µM)	0.8 µL	400 nM	
Template DNA	0.5-50 ng genomic	variable	
PCR grade dH_2O	Up to 20 µL final volume		

3. Program the instrument using following conditions, acquiring data on the FAM channel:

Cycles	Temperature	Time	Notes
1	95 ˚C	2 min	Polymerase activation, 2 minutes for cDNA and 3 minutes for genomic
45	95 ˚C 60 ˚C to 65 ˚C	5 seconds 20-30 seconds	Denaturation Annealing/Extension, do not exceed 30 seconds, do not use temperatures below 60 ˚C
HRM analysis	Refer to instrument instructions		