PCRBIOSYSTEMS
simplifying research

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Clara® HRM Mix

Product description

Clara® HRM Mix comprises our high-purity 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.

HRM analysis is a powerful technique for the analysis of mutations, polymorphisms and epigenetic differences in DNA samples. Clara® HRM Mix offers superior performance for accurate SNP discrimination and quantification of methylation differences.

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 -20 °C. Avoid prolonged exposure to light. If stored correctly, the kit will retain full activity until the indicated expiry date. The kit can be stored at 4 °C for 1 month.

Technical support

Scan or click the QR code for troubleshooting help and answers to frequently asked technical questions. For further technical support, please email technical@pcrbio.com with the following information:

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

TROUBLESHOOT

FAQS

Important considerations

Instrument compatibility: You can use our selection tool (https://pcrbio.com/resources/qpcr-selection-tool/) to identify compatible instruments. HRM can be carried out on any real time instrument with a high-resolution optical system able to detect small changes in fluorescence, the ability to perform precise temperature ramping at 0.1 to 0.3 ˚C increments, and software capable of analysing HRM data. If an instrument is not listed in our selection tool, please refer to your manufacturer's website or manual to ensure your instrument has these capabilities. If you are unsure, please contact technical@pcrbio.com.

Template: ≤1 µg genomic DNA, or ≤100 ng cDNA are recommended. However, users are encouraged to attempt a dilution series for new template/primer pairs to ensure that the PCR is efficient at that template concentration.

Primer design: For efficient amplification we recommend amplicon lengths between 80-200 bp. Shorter amplicons allow for faster cycling. Primers should have an approximate Tm of ~60 ˚C using default Primer 3 settings (https://bioinfo.ut.ee/primer3/). To verify the best annealing temperature for your primers in our products, please visit https://pcrbio.com/resources/tm-calculator/.

Reaction setup

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

Reagent	20 µL 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	≤0.1 µg genomic, ≤100 ng cDNA	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
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	See important considerations		