PCRBIOSYSTEMS
simplifying research

www.pcrbio.com

# qPCRBIO HRM Mix

## Product description:

Combined with the latest advancements in polymerase technology and advanced buffer chemistry, qPCRBIO HRM Mix offers market-leading accuracy in High Resolution Melt (HRM) analysis. qPCRBIO HRM Mix uses SyGreen 2, a 3rd generation, saturating, intercalating dye which does not inhibit PCR.

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

qPCRBIO HRM Mix uses antibody-mediated hot start technology that prevents the formation of primer-dimers to improve reaction sensitivity and specificity.

High-throughput screening has resulted in a buffer system that allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions.

| Pack Size | 2x qPCRBIO HRM Mix |
|---|---|
| 100 reactions | 1 x 1mL |
| 500 reactions | 5 x 1mL |
| 2000 reactions | 20 x 1mL |

## 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

The product may be used only for in vitro research purposes.

## Technical support

Help and support is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. 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®Nano |

## 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 qPCRBIO HRM Mix.
2. Prepare a master mix based on following table:

| Reagent | 20µL reaction | Final concentration | Notes |
|---|---|---|---|
| 2x qPCRBIO HRM Mix | 10µL | 1x | |
| Forward primer (10µM) | 0.8µL | 400nM | See above for optimal primer design |
| Reverse primer (10µM) | 0.8µL | 400nM | |
| Template DNA | <100ng cDNA, <1µg genomic | variable | See above for template considerations |
| PCR grade $dH_2O$ | Up to 20µL final volume | | |

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

| Cycles | Temperature | Time | Notes |
|---|---|---|---|
| 1 | 95˚C | 2min | Polymerase activation, 2 minutes for cDNA and 3 minutes for genomic |
| 40 | 95˚C<br>60˚C to 65˚C | 5 seconds<br>20-30 seconds | Denaturation<br>Anneal/Extension, do not exceed 30 seconds, do not use temperatures below 60˚C |
| HRM analysis | Refer to instrument instructions | | Optional melt profile analysis |