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2x 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. The kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

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

Technical support

For technical support and troubleshooting please email technical@pcrbio.com 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 (http://frodo.wi.mit.edu/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 60˚C to 65˚C	5 seconds 20-30 seconds	Denaturation Anneal/Extension, do not exceed 30 seconds, do not use temperatures below 60˚C
HRM analysis	Refer to instrument instructions		Optional melt profile analysis

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