PCRBIOSYSTEMS
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2x qPCRBIO Genotyping Mix No-ROX

Product description:

Combined with the latest advancements in polymerase technology and advanced buffer chemistry, qPCRBIO Genotyping Mix offers market-leading performance with minimal optimisation.

qPCRBIO Genotyping Mix is a kit designed for use in dual-labeled probe based genotyping assays including TaqMan®, Scorpions® and molecular beacon probe genotyping. qPCRBIO Genotyping Mix is fully compatible with ABI TaqMan® Pre-Designed SNP Genotyping assays.

qPCRBIO Genotyping 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 Genotyping Mix No-ROX
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. If stored correctly the kit will retain full activity for 12 months. 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

Important considerations

Instrument compatibility: Different real-time PCR instruments require different levels of ROX passive reference. Generally, modern instruments do not require passive reference but include the option to use it for normalisation. Please check our qPCRBIO Selection Table to determine which ROX concentration your instrument requires (http://www.pcrbio.com/realtime-pcr.html).

Primer design: For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80bp and 200bp. Amplicon lengths should not exceed 400bp. Primers should have a predicted melting temperature of around 60˚C, using default Primer 3 settings, 3mM $MgCl_2$ (http://frodo.wi.mit.edu/primer3/). For TaqMan® probes choose probe close to 5' primer, avoid terminal guanosine residues. The probe Tm should be approximately 10˚C higher than the primer Tm.

Template: Use between 1 and 20pg human genomic DNA per reaction. For accurate allele calling similar amounts of template must be used in all wells of the same run.

Reaction setup

1. Before starting, briefly vortex 2x qPCRBIO Genotyping Mix.
2. Prepare a master mix based on following table:

Reagent	20µl reaction	Final concentration	Notes
2x qPCRBIO Genotyping Mix	10µl	1x	
Forward primer (10µM)	0.8µl	400nM	See above for optimal primer design
Reverse primer (10µM)	0.8µl	400nM	
Probe (10µM)	0.4µl	200nM	
Template DNA	1 to 20pg human 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 appropriate channel:

Cycles	Temperature	Time	Notes
1	95˚C	2min	Polymerase activation, 2 minutes for cDNA and 3 minutes for genomic
40	95˚C 55-60˚C	15 seconds 60 seconds	Denaturation Anneal/Extension, do not exceed 60 seconds, for initial experiments use 57˚C