PCRBIOSYSTEMS
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2x qPCRBIO Probe Blue Mix Lo-ROX

Product description:

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

PCR Biosystems real-time PCR probe mixes have been designed for use on a wide range of probe technologies including TaqMan®, Scorpions® and molecular beacon probes.

qPCRBIO Probe Blue 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.

A blue dye has been added to aid with pipetting. The dye does not interfere with DNA synthesis but will impact the intensity of some fluorescent probes.

Pack size	2x qPCRBIO Probe Blue Mix No-ROX
100 reactions	1 x 1ml
500 reactions	5 x 1ml
2000 reactions	20 x 1ml
5000 reactions	1 x 50ml bottle
5000 reactions	50 x 1ml tubes

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

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. 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/). For TaqMan® probes choose probe close to 5' primer, avoid terminal guanosine residues.

Template amount: For genomic DNA, 1µg or less is recommended. For cDNA, 100ng or less is 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.

Probe Intensity: qPCRBIO Probe Blue Mix will necessarily lower the fluorescent intensity from probes by absorbing light at both the excitation and emission wavelengths (see Table 1). However, the recomended probe concentration of 200nM has proven sufficient for detection on all instruments tested. If signal intensity is a concern, consider switching to a qPCRBIO Probe Mix without dye.

Table 1: Fluorescent intensity of selected probes in qPCRBIO Probe Blue Mix.

Fluorophore	Ex / Em (nm)	Signal loss
FAM	494 / 518	12%
HEX	535 / 556	55%
Texas Red	595 / 615	88%
Cy5	675 / 694	82%

Reaction setup

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

Reagent	20µl reaction	Final concentration	Notes
2x qPCRBIO Probe Blue 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	See above for probe considerations
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 the 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 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
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridisation probes only