PCRBIOSYSTEMS
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qPCRBIO Probe Blue Mix Separate-ROX

Product description:

qPCRBIO Probe Blue Mix is a 2x mix designed to give superior sensitivity and specificity in all probe-based real-time PCR assays, including TaqMan, Scorpions, and molecular beacon probes.

qPCRBIO Probe Blue Mix can be used to detect extremely low copy number targets and quantify any DNA template including genomic, cDNA and viral sequences. The enhanced sensitivity of the mix makes it the perfect choice for multiplexing.

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

A separate tube of 50 µM ROX additive is provided, enabling use on all real-time instruments.

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 qPCRBIO Probe Blue Mix No-ROX	50µM ROX Additive
100 reactions	1 x 1 mL	1 x 200 µL
500 reactions	5 x 1 mL	1 x 200 µL
2000 reactions	20 x 1 mL	4 x 200 µL
5000 reactions	1 x 50 mL bottle	2 x 520 µL
5000 reactions	50 x 1 mL tubes	2 x 520 µL

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

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at https://pcrbio.com/terms-conditions/.

Important considerations

Instrument compatibility: Different qPCR instruments may require different levels of ROX passive reference for normalisation. Use our qPCR Selection Tool to determine which ROX concentration your instrument requires (https://pcrbio.com/resources/qpcr-selection-tool/).

ROX additive: The 50 µM ROX Additive can be added directly to the 1 mL tube of mix supplied. Once added, the reagent may be used straight away or stored at -30 ˚C to -20 ˚C for future use. Use the table below to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

Reagent	Lo-ROX instruments	Hi-ROX instruments	Reaction concentration
2x qPCRBIO Probe Blue Mix No-ROX	1 mL	1 mL	1x
50 µM ROX Additive	4 µL	35 µL	100 nM (Lo-), 875 nM (Hi-ROX)

Primer design: For efficient amplification we recommend amplicon lengths between 80-200 bp and not exceeding 400 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/. For TaqMan probes choose a probe close to the 5' primer, avoiding terminal guanosine residues.

Template amount: The kit can be used with cDNA or DNA synthesised or extracted by most commercial kits or standard extraction methods, provided the amount and quality of template are within an acceptable range. Addition of 2 to 5 µL volumes of sample will improve assay precision. For genomic DNA, 1 µg or less is recommended. For cDNA, 100 ng 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 200 nM 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 following table:

Reagent	20 µL reaction	Final concentration	Notes
2x qPCRBIO Probe Blue Mix	10 µL	1x	Ensure ROX is added prior to this step
Forward primer (10 µM)	0.8 µL	400 nM	See above for optimal primer design
Reverse primer (10 µM)	0.8 µL	400 nM	
Probe (10 µM)	0.4 µL	200 nM	
Template DNA	<100 ng cDNA, <1 µg genomic	variable	See above for template considerations
PCR grade dH_2O	Up to 20 µL final volume		

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

Cycles	Temperature	Time	Notes
1	95 ˚C	2 min	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