PCRBIOSYSTEMS
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2x qPCRBIO SyGreen Blue Mix Separate-ROX

Product description:

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

PCR Biosytems SyGreen Mixes use an intercalating dye which does not inhibit PCR, unlike other popular dyes. A non-reactive blue dye has been added to assist researchers during pipetting.

qPCRBIO SyGreen 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.

Pack size	2x qPCRBIO SyGreen Blue Mix No-ROX	50µM ROX Additive
100 reactions	1 x 1ml	1 x 200µl
500 reactions	5 x 1ml	1 x 200µl
2000 reactions	20 x 1ml	4 x 200µl
5000 reactions	1 x 50ml bottle	2 x 520µl
5000 reactions	50 x 1ml tubes	2 x 520µl

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

50µM ROX Additive

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).

ROX additive protocol: The 50µM ROX Additive supplied is formulated to be added directly to the 1ml tube of 2x qPCRBIO master mix supplied. Once the ROX is added, the reagent may be used straight away or stored between -30˚C and -15˚C for future use. Please use the following charts to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

ROX for Hi-ROX instruments:

Reagent	Hi-ROX instruments	Final concentration	Reaction concentration
2x qPCRBIO SyGreen Blue Mix No-ROX	1.0ml	2x	1x
50µM ROX Additive	35.0µl	1.75µM	875nM

ROX for Lo-ROX instruments:

Reagent	Lo-ROX instruments	Final concentration	Reaction concentration
2x qPCRBIO SyGreen Blue Mix No-ROX	1.0ml	2x	1x
50µM ROX Additive	4.0µl	200nM	100nM

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/).

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.

Reaction setup

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

Reagent	20µl reaction	Final concentration	Notes
2x qPCRBIO SyGreen Blue 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 the 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
Melt analysis	Refer to instrument instructions		Optional melt profile analysis