



PCRBIO SYSTEMS
simplifying research

2x qPCR BIO Probe Mix Hi-ROX

www.pcrbio.com

Product description:

Combined with the latest advancements in polymerase technology and advanced buffer chemistry qPCR BIO Probe 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.

qPCR BIO Probe 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 qPCR BIO Probe Mix Hi-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.

Reaction setup

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

| Reagent | 20µl reaction | Final concentration | Notes |
|-----------------------------|---------------------------|---------------------|---------------------------------------|
| 2x qPCRBIO Probe 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 | <100ng cDNA, <1µg genomic | variable | See above for template considerations |
| PCR grade dH ₂ O | 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 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 |