



**PCRBIO SYSTEMS**  
simplifying research

## 2x qPCR BIO Probe Blue Mix Lo-ROX

### Product description:

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

qPCR BIO 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 qPCR BIO 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                          |
| 5000 reactions | 50 x 1ml                          |

### Shipping and storage

On arrival the kit should be stored at -20°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](mailto:technical@pcrbio.com) the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile

[www.pcrbio.com](http://www.pcrbio.com)

## 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 qPCR BIO 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:** qPCR BIO 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 recommended probe concentration of 200nM has proven sufficient for detection on all instruments tested. If signal intensity is a concern, consider switching to a qPCR BIO Probe Mix without dye.

**Table 1:** Fluorescent intensity of selected probes in qPCR BIO 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 qPCR BIO Probe Blue Mix.
2. Prepare a master mix based on the following table:

| Reagent                     | 20µl reaction             | Final concentration | Notes                                 |
|-----------------------------|---------------------------|---------------------|---------------------------------------|
| 2x qPCR BIO 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 <sub>2</sub> O | 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<br>60°C to 65°C             | 5 seconds<br>20-30 seconds | Denaturation<br>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                        |