**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.

<table>
<thead>
<tr>
<th>Pack size</th>
<th>2x qPCRBIO SyGreen Blue Mix No-ROX</th>
<th>50µM ROX Additive</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 reactions</td>
<td>1 x 1mL</td>
<td>1 x 200µL</td>
</tr>
<tr>
<td>500 reactions</td>
<td>5 x 1mL</td>
<td>1 x 200µL</td>
</tr>
<tr>
<td>2000 reactions</td>
<td>20 x 1mL</td>
<td>4 x 200µL</td>
</tr>
<tr>
<td>5000 reactions</td>
<td>1 x 50mL bottle</td>
<td>2 x 520µL</td>
</tr>
<tr>
<td>5000 reactions</td>
<td>50 x 1mL tubes</td>
<td>2 x 520µL</td>
</tr>
</tbody>
</table>

**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.

**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**

**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 ([https://bioinfo.ut.ee/primer3/](https://bioinfo.ut.ee/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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>20µL reaction</th>
<th>Final concentration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x qPCRBIO SyGreen Blue Mix</td>
<td>10µL</td>
<td>1x</td>
<td></td>
</tr>
<tr>
<td>Forward primer (10µM)</td>
<td>0.8µL</td>
<td>400nM</td>
<td>See above for optimal primer design</td>
</tr>
<tr>
<td>Reverse primer (10µM)</td>
<td>0.8µL</td>
<td>400nM</td>
<td></td>
</tr>
<tr>
<td>Template DNA</td>
<td>&lt;100ng cDNA, &lt;1µg genomic</td>
<td>Variable</td>
<td>See above for template considerations</td>
</tr>
<tr>
<td>PCR grade dH₂O</td>
<td>Up to 20µL final volume</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Temperature</th>
<th>Time</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95°C</td>
<td>2min</td>
<td>Polymerase activation, 2 minutes for cDNA and 3 minutes for genomic</td>
</tr>
<tr>
<td>40</td>
<td>95°C</td>
<td>5 seconds</td>
<td>Denaturation, Anneal/Extension, do not exceed 30 seconds, do not use temperatures below 60°C</td>
</tr>
<tr>
<td></td>
<td>60°C to 65°C</td>
<td>20-30 seconds</td>
<td></td>
</tr>
</tbody>
</table>

**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 Tool to determine which ROX concentration your instrument requires ([https://pcrbio.com/resources/qpcr-selection-tool/](https://pcrbio.com/resources/qpcr-selection-tool/)).

**ROX additive protocol:** The 50µM ROX Additive supplied is formulated to be added directly to the 1ml tube of 2x qPCRmix 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:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Hi-ROX instruments</th>
<th>Final concentration</th>
<th>Reaction concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x qPCRBIO SyGreen Blue Mix</td>
<td>1.0mL</td>
<td>2x</td>
<td>1x</td>
</tr>
<tr>
<td>50µM ROX Additive</td>
<td>35.0µL</td>
<td>1.75µM</td>
<td>875nM</td>
</tr>
</tbody>
</table>

**ROX for Lo-ROX instruments:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Lo-ROX instruments</th>
<th>Final concentration</th>
<th>Reaction concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x qPCRBIO SyGreen Blue Mix</td>
<td>1.0mL</td>
<td>2x</td>
<td>1x</td>
</tr>
<tr>
<td>50µM ROX Additive</td>
<td>4.0µL</td>
<td>200nM</td>
<td>100nM</td>
</tr>
</tbody>
</table>