Product description

PCRBIO Taq Mix Red uses the latest developments in polymerase technology and buffer chemistry to enhance PCR speed, yield and specificity. The enzyme and buffer system allow for superior PCR performance on complex templates such as mammalian genomic DNA. Reactions can be directly loaded onto agarose gels without additional loading buffer.

PCRBIO Taq Mix Red is a robust mix for all your everyday PCR applications including genotyping, screening and library construction. This 2x mix contains PCRBIO Taq DNA Polymerase which performs consistently well on a broad range of templates (including both GC and AT-rich).

PCRBIO Taq DNA Polymerase has an error rate of approximately 1 error per $2.0 \times 10^5$ nucleotides incorporated. PCR products generated with PCRBIO Taq DNA Polymerase are A-tailed and may be cloned into TA cloning vectors.

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.

Component | 200 reactions | 1000 reactions
--- | --- | ---
2x PCRBIO Taq Mix Red | 5 x 1mL | 25 x 1mL

Shipping and storage

On arrival the kit should be stored at 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 for in vitro research purposes only.

Technical support

Help and support is available on our website at [https://pcrbio.com/resources/](https://pcrbio.com/resources/) including answers to frequently asked technical questions. For technical support and troubleshooting you can submit a technical enquiry online, or alternatively email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of gel images
Important considerations

2x PCRBIOTaq Mix Red: The 2x mix contains PCRBIO Taq DNA Polymerase, 6mM MgCl$_2$, 2mM dNTPs, enhancers, stabilizers and a red dye for tracking during agarose electrophoresis. It is not recommended to add further PCR enhancers or MgCl$_2$ to the reaction. The buffer composition has been optimised to maximise PCR success rates.

Template: For eukaryotic DNA use between 5ng and 500ng per reaction, for cDNA use below 100ng per reaction.

Primers: Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings ([http://bioinfo.ut.ee/primer3/](http://bioinfo.ut.ee/primer3/)). The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 55°C annealing temperature then increase in 2°C increments if non-specific products are present.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity of template. 20 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1kb and 6kb. For shorter amplicons, faster cycling is possible.

Agarose gel electrophoresis dye migration: The 2x mix contains a red dye for tracking during agarose gel electrophoresis. In a 2% agarose TAE gel the dye migrates at a rate equivalent to 350bp of DNA. In a 1% agarose TAE gel the dye migration rate is equivalent to 600bp of DNA.

Reaction setup

1. Prepare a master mix based on the following table:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>50µL reaction</th>
<th>Final concentration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x PCRBIOTaq Mix Red</td>
<td>25.0µL</td>
<td>1x</td>
<td></td>
</tr>
<tr>
<td>Forward primer (10µM)</td>
<td>2.0µL</td>
<td>400nM</td>
<td>See above for optimal primer design</td>
</tr>
<tr>
<td>Reverse primer (10µM)</td>
<td>2.0µL</td>
<td>400nM</td>
<td></td>
</tr>
<tr>
<td>Template DNA</td>
<td>&lt;100ng cDNA, &lt;500ng genomic</td>
<td>variable</td>
<td>See above for template considerations</td>
</tr>
<tr>
<td>PCR grade dH$_2$O</td>
<td>Up to 50µL final volume</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Cycle using conditions based on the following table:

<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>1min</td>
<td>Initial denaturation</td>
</tr>
<tr>
<td>40</td>
<td>95°C</td>
<td>15 seconds</td>
<td>Denaturation</td>
</tr>
<tr>
<td></td>
<td>55°C to 65°C</td>
<td>15 seconds</td>
<td>Anneal</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>1 to 120 seconds</td>
<td>Extension (20 seconds per kb)</td>
</tr>
</tbody>
</table>