

# 2x PCRBIO Taq Mix Red

### 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.

2x PCRBIO Taq Mix Red is a robust mix for all your everyday PCR applications including genotyping, screening and library construction.

PCRBIO Taq DNA Polymerase can perform 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 x 10<sup>5</sup> 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		1000 reactions
2x PCRBIO Taq Mix Red	5x 1ml	25x 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 the following information:

Amplicon size Reaction setup Cycling conditions Screen grabs of gel images



#### Important considerations

2x PCRBIO Taq 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://frodo.wi.mit.edu/primer3/). The final primer concentration in the reaction should be between  $0.2\mu$ M and  $0.6\mu$ M.

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. 15 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1kb and 6kb. For shorter amplicons a 1 second extension is sufficient.

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:

Reagent	50µl reaction	Final concentration	Notes	
2x PCRBIO Taq Mix Red	25.0µl	lx		
Forward primer (10µM)	2.0µl 400nM See abo		See above for optimal	
Reverse primer (10µM)	2.0µl	400nM	primer design	
Template DNA	<100ng cDNA, <500ng genomic variable		See above for template considerations	
PCR grade dH <sub>2</sub> O	Up to 50µl final volume			

#### 2. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	95°C	lmin	Initial denaturation
40	95°C 55°C to 65°C 72°C	15 seconds 15 seconds 1 to 90 seconds	Denaturation Anneal Extension (15 seconds per kb)