PCRBIOSYSTEMS
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PCRBIO Ultra Mix Red

Product description:

PCRBIO Ultra Mix Red has been engineered for the amplification of extremely difficult templates, with the convenience of direct loading onto agarose gels without the need for additional loading buffer. The latest polymerase developments are combined with antibody-mediated hot start technology to deliver outstanding performance for all your PCR applications.

PCRBIO Ultra Mix Red is powered by PCRBIO Ultra Polymerase, a highly robust enzyme designed for efficient and reliable amplification of challenging and complex targets, even under difficult conditions such as the presence of inhibitors. The enzyme and buffer system have been developed to give superior PCR performance and higher success rates on a broad range of templates, including complex genomic DNA and targets with a high GC content.

Our antibody-mediated hot start formulation prevents the formation of primer dimers and non-specific products, allowing for specific and sensitive amplification from low copy number target sequences.

PCRBIO Ultra Mix Red has an error rate of approximately 1 error per 5.0 x 10^5 nucleotides incorporated. PCR products are A-tailed and may be cloned into TA cloning vectors.

Component	80 reactions	400 reactions
2x PCRBIO Ultra Mix Red	2 x 1 ml	10 x 1 ml

Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. 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 for in vitro research purposes only.

Technical support

Help and support is available on our website at 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 siz
- Reaction setup
- Cycling conditions
- Screen grabs of gel images

Important considerations

2x PCRBIO Ultra Mix Red: The 2x mix contains PCRBIO Ultra DNA Polymerase, 6 mM $MgCl_2$, 2 mM 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 5 ng and 500 ng per reaction, for cDNA use below 100 ng per reaction.

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

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 below 5 kb. For amplicons greater than 5 kb we recommend between 40 and 60 seconds per kb.

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 50-100 bp of DNA. In a 1% agarose TAE gel the dye migration rate is equivalent to 200-300 bp of DNA.

Reaction setup

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

Reagent	50 µL reaction	Final concentration	Notes
2x PCRBIO Ultra Mix Red	25.0 µL	1x	
Forward primer (10 µM)	2.0 µL	400 nM	See above for optimal primer design
Reverse primer (10 µM)	2.0 µL	400 nM	
Template DNA	<100 ng cDNA, <500 ng genomic	Variable	See above for template considerations
PCR grade dH_2O	Up to 50 µL final volume		

2. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	95 ˚C	1 min to 2 min	Initial denaturation and enzyme activation
40	95 ˚C	15 seconds	Denaturation
	55 ˚C to 65 ˚C	15 seconds	Anneal
	72 ˚C	10 minutes*	Extension (50 seconds per kb). *See notes above.