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PCRBIO HS Taq Mix Red

Product description

PCRBIO HS Taq Mix Red uses the latest developments in polymerase technology and buffer chemistry to enhance PCR speed, yield and specificity. The enzyme uses advanced hot start technology for superior sensitivity. Reactions can be directly loaded onto agarose gels without additional loading buffer.

The enzyme and buffer system allow for superior PCR performance on complex templates such as mammalian genomic DNA. Due to enhanced efficiency and specificity the enzyme is perfectly suited to difficult PCR.

The mix contains PCRBIO HS Taq DNA Polymerase, a robust enzyme ideal for all your everyday PCR applications including genotyping, screening, library construction and multiplex PCR. The enzyme is particularly resistant to PCR inhibitors and is suitable for direct PCR from unprocessed samples including bacterial culture, bacterial colonies, blood and urine.

PCRBIO HS Taq Mix Red has an error rate of approximately 1 error per 2.0 x 10^5 nucleotides incorporated. PCR products generated with PCRBIO HS 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 both fast and standard cycling conditions.

Component	200 reactions	1000 reactions
2x PCRBIO HS Taq Mix Red	5 x 1 mL	25 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 size
- Reaction setup
- Cycling conditions
- Screen grabs of gel images

Important considerations

2x PCRBIO HS Taq Mix Red: The 2x mix contains PCRBIO HS Taq 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 (http://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. 20 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1 kb and 6 kb. For shorter amplicons, faster cycling is possible.

Multiplex PCR: When first performing multiplex PCR it is recommended to run an annealing temperature gradient from 55 ˚C to 65 ˚C. The annealing temperature that results in the best specificity should be used in subsequent experiments. Fast cycling conditions should not be used for multiplex PCR. Initially, we recommend a 90 second extension time. This time may be further extended to increase yield.

Colony PCR: From bacterial colonies use a sterile tip to pick a colony and resuspend into a 50 µL reaction as described below. From liquid culture add 5 µL of overnight culture to the final mix.Increase initial denaturation time to 10 minutes.

Direct blood/urine PCR: Add 2 µL mammalian blood or urine to a 50 µL reaction as described below.

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 HS Taq 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	<100ng 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. For colony PCR increase denaturation time to 10 minutes
40	95 ˚C 55 ˚C to 65 ˚C 72 ˚C	15 seconds 15 seconds 1 to 120 seconds	Denaturation Anneal Extension (20 seconds per kb). For multiplex PCR use 90 seconds