PCRBIOSYSTEMS
simplifying research

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2x 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. 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 multiplex PCR. Reactions can be directly loaded onto agarose gels without additional loading buffer.

2x PCRBIO HS Taq Mix Red is a robust mix for all your everyday PCR applications including genotyping, multiplex PCR, screening, library construction, colony PCR and PCR direct from blood and urine. PCRBIO HS Taq DNA Polymerase can perform consistently well on a broad range of templates (including both GC and AT rich).

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.

Component	200 reactions	1000 reactions
2x PCRBIO HS Taq Mix Red	5 x 1ml	25 x 1ml

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.

PCRBIO HS Taq Mix Red is particularly resistant to PCR inhibitors. The mix is suitable for direct PCR from unprocessed samples including bacterial culture, bacterial colonies, blood and urine.

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. 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

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Important considerations

2x PCRBIO HS Taq Mix Red: The 2x mix contains PCRBIO HS 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 0.2µM 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. 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.

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 50µl reaction as described below. From liquid culture add 5µl of overnight culture to the final mix.Increase initial denaturation time to 10 minutes.

Direct blood/urine PCR: Add 2µl mammalian blood or urine to a 50µl 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 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 HS Taq Mix Red	25.0µl	1x	
Forward primer (10µM)	2.0µl	400nM	See above for optimal primer design
Reverse primer (10µM)	2.0µl	400nM	
Template DNA	<100ng cDNA, <500ng genomic	variable	See above for template considerations
PCR grade dH_2O	Up to 50µl final volume		

2. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	95˚C	1min to 2min	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 90 seconds	Denaturation Anneal Extension (15 seconds per kb). For multiplex PCR use 90 seconds