PCRBIOSYSTEMS
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PCRBIO HiFi Polymerase

Product description:

PCRBIO HiFi Polymerase 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 high-fidelity PCR performance on complex templates such as mammalian genomic DNA.

PCRBIO HiFi is a robust enzyme for all your everyday PCR applications including genotyping, screening and library construction. PCRBIO HiFi Polymerase can perform consistently well on a broad range of templates (including both GC and AT rich).

PCRBIO HiFi Polymerase has an error rate of approximately 1 error per 4.5 x 10^7 nucleotides incorporated. This is over 50 times lower than taq DNA polymerase. The polymerase has 3' to 5' exonuclease activity.

Component	200 units	1000 units
PCRBIO HiFi Polymerase (2u/µl)	1x 100µl	5x 100µl
5x PCRBIO HiFi buffer	3x 1ml	15x 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

PCRBIO 5x Reaction Buffer: The 5x reaction buffer contains 15mM $MgCl_2$, 5mM dNTPs, enhancers and stabilizers. 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 57˚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. 30 seconds per kilobase(kb) is recommended for amplification from eukaryotic genomic DNA or cDNA.

Reaction setup

1. Allow 5x PCRBIO HiFi Buffer to reach room temperature, briefly vortex.
2. Prepare a master mix based on the following table:

Reagent	50µl reaction	Final concentration	Notes
5x PCRBIO Reaction Buffer	10.0µl	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
PCRBIO HiFi Polymerase (2u/µl)	0.5µl		
PCR grade dH_2O	Up to 50µl final volume		

3. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	95˚C	1min	Initial denaturation
25-35	95˚C 55˚C to 65˚C 72˚C	15 seconds 15 seconds 30 seconds per kb	Denaturation Anneal Extension (30 seconds per kb)