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PCRBIO Ultra Polymerase

Product description:

PCRBIO Ultra Polymerase has been engineered for the amplification of extremely difficult templates. The latest polymerase developments are combined with antibody-mediated hot start technology to deliver outstanding performance for all you PCR applications.

PCRBIO Ultra Polymerase is 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.

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

Component	250 units	1000 units
PCRBIO Ultra Polymerase (5u/µl)	1 x 50µl	4 x 50µl
5x PCRBIO Ultra Buffer	2 x 1ml	8 x 1ml

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 with the following information:

Amplicon size
Reaction setup
Cycling conditions
Screen grabs of gel images

Important considerations

PCRBIO 5x Ultra Buffer: The 5x Ultra 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. 15 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons below 5kb. For amplicons greater than 5kb we recommend between 40 and 60 seconds per kb.

Reaction setup

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

Reagent	50µl reaction	Final concentration	Notes
5x Ultra 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 Ultra Polymerase (5u/µl)	0.25µl to 1.0µl		
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 to 2min	Initial denaturation and enzyme activation
25-35	95˚C 55˚C to 65˚C 72˚C	15 seconds 15 seconds 10 minutes*	Denaturation Anneal Extension (50 seconds per kb). *See notes above.

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