PCRBIOSYSTEMS
simplifying research

PCRBIO Classic Taq

Product description:

PCRBIO Classic Taq is a highly-purified, recombinant Taq DNA Polymerase. The latest developments in buffer chemistry allow for enhanced PCR speed, yield and specificity. The enzyme and buffer system allow for superior PCR performance on complex templates such as mammalian genomic DNA. For added convenience the 10x PCRBIO Classic Buffer contains 30mM $MgCl_2$.

PCRBIO Classic Taq is a robust enzyme for all your everyday PCR applications including genotyping, screening and library construction. PCRBIO Taq DNA Polymerase performs consistently well on a broad range of templates (including both GC and AT rich). PCRBIO Classic Taq has 5'-3' exonuclease activities, but no 3'-5' exonuclease (proofreading) activity.

The enzyme has an error rate of approximately 1 error per 2.0 x 10^5 nucleotides incorporated. PCR products generated with PCRBIO Classic Taq are A-tailed and may be cloned into TA cloning vectors.

Component	500 units	1000 units	2000 units
PCRBIO Classic Taq (5u/µl)	1 x 100µl	2 x 100µl	4 x 100µl
10x PCRBIO Classic Buffer + 30mM MgCl2	2 x 1ml	4 x 1ml	8 x 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

10x PCRBIO Classic Buffer: The 10x reaction buffer contains enhancers, stabilizers and 30mM $MgCl_2$. It is not recommended to add further PCR enhancers to the reaction. The buffer composition has been optimised to maximise PCR success rates.

$MgCl_2$ and dNTPs: A final reaction concentration of 3mM $MgCl_2$ to 1mM dNTPs (0.25mM each) is recommended. 30mM $MgCl_2$ is included in the 10x PCRBIO Classic Buffer. Additional $MgCl_2$ is not necessary.

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.

Reaction setup

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

Reagent	50µl reaction	Final concentration	Notes
10x PCRBIO Classic Buffer + 30mM $MgCl_2$	5.0µl	1x Buffer, 3mM $MgCl_2$	
100mM dNTPs (25mM each)	0.5µl	1mM (0.25mM each)	
Forward primer (10µM)	2.0µl	400nM	See above for primer design considerations
Reverse primer (10µM)	2.0µl	400nM	
Template DNA	<100ng cDNA, <500ng genomic	variable	See above for template considerations
PCRBIO Classic Taq (5u/µl)	0.25µl - 1.0µl		
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	Initial denaturation
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)