



PCRBIOSYSTEMS
simplifying research

2x PCRBIO Ultra Mix

Product description:

PCRBIO Ultra Mix has been engineered for the amplification of extremely difficult templates and long amplicons. Latest polymerase developments are combined with PCR Biosystems proprietary hot-start technology to deliver outstanding performance for all your PCR applications.

2x PCRBIO Ultra Mix uses the latest developments in DNA polymerase technology and buffer chemistry to enhance PCR speed, yield and specificity. The enzyme and buffer system allow for superior PCR performance on complex templates such as mammalian genomic DNA and amplicons up to 35kb. PCRBIO Ultra Polymerase can perform consistently well on a broad range of templates (including both GC and AT rich).

PCRBIO Ultra Mix has an error rate of approximately 1 error per 5.0×10^5 nucleotides incorporated. PCR products generated with PCRBIO Ultra DNA Polymerase are A-tailed and may be cloned into TA cloning vectors.

Component	80 reactions	400 reactions
2x PCRBIO Ultra Mix	2x 1ml	10x 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 the following information to:

technical@pcrbio.com

Amplicon size
Reaction setup
Cycling conditions
Screen grabs of gel images

www.pcrbio.com

Important considerations

2x PCRBIO Ultra Mix: The 2x mix contains PCRBIO Ultra DNA Polymerase, 6mM MgCl₂, 2mM dNTPs, enhancers and stabilizers. It is not recommended to add further PCR enhancers or MgCl₂ 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 below 5kb. For amplicons between 5kb and 35kb we recommend between 40 and 60 seconds per kb.

Reaction setup

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

Reagent	50µl reaction	Final concentration	Notes
2x PCRBIO Ultra Mix	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 ₂ O	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
40	95°C	15 seconds	Denaturation
	55°C to 65°C	15 seconds	Anneal
	72°C	10 minutes*	Extension (50 seconds per kb). *See notes above.