



**PCRBIOSYSTEMS**  
simplifying research

## PCRBIO HiFi Polymerase

[www.pcrbio.com](http://www.pcrbio.com)

### 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  $4.5 \times 10^7$  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/ $\mu$ L)	1 x 100 $\mu$ L	5 x 100 $\mu$ L
5x PCRBIO HiFi Buffer	3 x 1mL	15 x 1mL

### Shipping and storage

On arrival the kit should be stored between -30°C and -15°C. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month.

### Limitations of product use

The product may be used for in vitro research purposes only.

### Technical support

Help and support is available on our website at <https://pcrbio.com/resources/> including answers to frequently asked technical questions. For technical support and troubleshooting you can submit a technical enquiry online, or alternatively email [technical@pcrbio.com](mailto:technical@pcrbio.com) with 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<sub>2</sub>, 5mM dNTPs, enhancers and stabilizers. It is not recommended to add further PCR enhancers or MgCl<sub>2</sub> 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 (<https://bioinfo.ut.ee/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 <sub>2</sub> O	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	15 seconds	Denaturation
	55°C to 65°C	15 seconds	Anneal
	72°C	30 seconds per kb	Extension (30 seconds per kb)