PCRBIOSYSTEMS
simplifying research

PCRBIO HS VeriFi™ Polymerase

Product description:

PCRBIO HS VeriFi™ Polymerase is a robust and versatile proofreading enzyme with AptaLock™ hot start technology for highly precise PCR. The enzyme is designed for all PCR applications where greater sequence accuracy is required, together with improved PCR success rates of long and challenging templates.

PCRBIO HS VeriFi™ Polymerase is derived from Pfu DNA polymerase for its 3'-5' exonuclease (proofreading) activity. The enzyme is engineered with proprietary mutations that significantly increase processivity, resulting in shorter extension times (30 s/kb), higher yields and the ability to amplify longer and more difficult targets, including eukaryotic genomic templates in excess of 17.5kb.

PCRBIO's innovative AptaLock™ technology uses a proprietary aptamer-like molecule that reversibly inhibits both the 3'-5' exonuclease activity and 5'-3' polymerase activity of the enzyme at ambient temperatures. This unique hot start molecule prevents primer dimer formation and non-specific amplification to maximize the sensitivity and specificity of your PCR. This feature makes the enzyme highly suitable for multiplexing and enables reactions to be set up at room temperature.

The enhanced accuracy of PCRBIO HS VeriFi™ Polymerase results in fidelity that is approximately 100 times higher than Taq DNA polymerase. The enzyme is ideal for applications where greater accuracy is needed, such as cloning, site-directed mutagenesis and sequencing. PCR products generated with this range of products are blunt ended.

Component	100 units	500 units
PCRBIO HS VeriFi™ Polymerase (2u/µL)	1 x 50µL	1 x 250µL
5x PCRBIO VeriFi™ Buffer	1 x 1.7mL	3 x 1.7mL
10x VeriMax Enhancer	1 x 1.7mL	2 x 1.7mL

PCRBIO HS VeriFi™ Polymerase is provided with an advanced buffer system including dNTPs, Mg and enhancers, enabling high fidelity PCR of a wide range of targets and fragment sizes regardless of GC content.

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 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 with the following information:

Amplicon size
Reaction setup
Cycling conditions
Screen grabs of gel images

Important considerations

5x PCRBIO VeriFi™ Buffer: The 5x buffer contains 15mM $MgCl_2$, 5mM dNTPs, enhancers and stabilizers. It is not recommended to add further $MgCl_2$ to the reaction. The buffer composition has been optimized to maximize PCR success rates.

Reaction Enhancer: In situations where no amplification is observed, we recommend adding the 10x VeriMax Enhancer to the reaction mix. This enhancer can improve the performance of PCRBIO HS VeriFi™ Polymerase on some difficult or long templates, for example GC-rich templates or those with complex secondary structures.

Primers: Primers should have a predicted melting temperature of around 60˚C, using default Primer 3 settings (http://bioinfo.ut.ee/primer3/). The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Denaturation: Denaturation should be performed at 95˚C. However, if the presence of high GC regions results in low yields, increasing the melting temperature to 98-100˚C can improve the amount of product.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 60˚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 most applications. Two-step cycling protocols may also be used with combined annealing and extension at 68-75˚C.

Multiplex PCR: The optimal extension time for multiplex reactions will be dependent on the complexity of template, the length of amplicons, and the number of targets. We recommend starting with the extension time of the longest fragment, and then increasing in increments of between 10 and 30 seconds if necessary.

Reaction setup

1. Allow 5x PCRBIO VeriFi™ Buffer (and 10x VeriMax Enhancer, if used) to reach room temperature, then briefly vortex.

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

Reagent	25µL reaction	50µL reaction	Final concentration	Notes
5x PCRBIO VeriFi™ Buffer	5.0µL	10.0µL	1x	
10x VeriMax Enhancer (optional)	2.5µL	5.0µL	1x	See above for use of enhancer
Forward primer (10µM)	1.0µL	2.0µL	400nM	See above for optimal primer design
Reverse primer (10µM)	1.0µL	2.0µL	400nM	
Template DNA	<100ng genomic DNA <5ng less complex DNA	<200ng genomic DNA <10ng less complex DNA	variable	
PCRBIO HS VeriFi™ Polymerase (2u/µL)	0.25µL	0.5µL		
PCR grade dH_2O	Up to 25µL final volume	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 60˚C to 75˚C 72˚C	15 seconds 15 seconds 30 seconds / kb	Denaturation (see above for high GC templates) Anneal Extension (see above for multiplex PCR)