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VeriFi™ Polymerase

Product description:

VeriFi™ Polymerase is a versatile and robust high fidelity enzyme engineered for PCR applications where greater sequence accuracy is required, together with improved PCR success rates of long and challenging templates.

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 (10-30 s/kb), higher yields and the ability to amplify longer and more difficult targets, including eukaryotic genomic templates in excess of 17.5 kb.

The high accuracy and enhanced 3'-5' exonuclease activity of VeriFi™ Polymerase result in fidelity that is approximately 100 times higher than Taq DNA polymerase. The enzyme is ideally suited to applications where greater accuracy is required, such as cloning, site-directed mutagenesis and sequencing. PCR products generated with this range of products are blunt ended.

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 with minimal or no optimisation required.

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

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 with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of gel images

Important considerations

5x VeriFi™ Buffer: The 5x buffer contains 15 mM $MgCl_2$, 5 mM dNTPs, enhancers and stabilizers. It is not recommended to add further $MgCl_2$ to the reaction. The buffer composition has been optimised to maximise 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 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 0.2 µM and 0.6 µM.

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 however shorter extension times of between 10 and 30 seconds per kb are possible. Two-step cycling protocols may also be used with combined annealing and extension at 68-75 ˚C.

Fast cycling: If using faster extension times, care must be taken to prevent loading too much template DNA. If non-specific bands are visible after amplification, the amount of template DNA should be decreased.

Reaction setup

1. Allow 5x VeriFi™ Buffer (and 10x VeriMax Enhancer, if used) to reach room temperature, then briefly vortex.
2. Prepare a master mix on ice based on the following table:

Reagent	25µL reaction	50µL reaction	Final concentration	Notes
5x VeriFi™ Buffer	5.0 µL	10.0 µL	1x	
10x VeriMax Enhancer (Optional)	2.5 µL	5.0 µL	1x	See above for use of enhancer
Forward primer (10µM)	1.0 µL	2.0 µL	400 nM	See above for optimal primer design
Reverse primer (10µM)	1.0 µL	2.0 µL	400 nM	
Template DNA	<100 ng genomic DNA <5 ng less complex DNA	<200 ng genomic DNA <10 ng less complex DNA	variable	
VeriFi™ Polymerase (2u/µL)	0.25 µL	0.5 µL		
PCR grade dH_2O	Up to 25 µL final volume	Up to 50 µL final volume		

3. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	95 ˚C	1 min	Initial denaturation
25-35	95 ˚C	15 seconds	Denaturation (see above for high GC templates)
	60 ˚C to 75 ˚C	15 seconds	Anneal
	72 ˚C	10-30 seconds/kb	Extension (see above for optimal extension time and fast cycling considerations)