PCRBIOSYSTEMS
simplifying research

www.pcrbio.com

VeriFi™ Hot Start Mix Red

Product description:

VeriFi™ Hot Start Mix Red is a convenient high fidelity mix with AptaLock™ hot start technology for highly precise PCR. This 2x ready mix is designed for PCR applications where greater sequence accuracy is required, together with improved PCR success rates of long and challenging templates. The mix contains a red dye suitable for direct loading and tracking during agarose gel electrophoresis.

VeriFi™ Hot Start Mix Red contains the highly processive VeriFi™ Hot Start Polymerase, developed for robust and versatile high fidelity PCR. The enzyme is derived from Pfu DNA polymerase for its 3'-5' exonuclease (proofreading) activity. Several proprietary mutations significantly improve DNA binding and 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 17.5 kb.

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 maximise 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 VeriFi™ Hot Start Polymerase results in fidelity that is approximately 100 times higher than Taq DNA polymerase, making it ideal for applications such as cloning, site-directed mutagenesis and sequencing.

Component	100 x 50 µL rxns	500 x 50 µL rxns
2x VeriFi™ Hot Start Mix Red	2 x 1.25 mL	10 x 1.25 mL

VeriFi™ Hot Start Mix Red uses 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. PCR products generated with this range of products are blunt ended.

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

2x VeriFi™ Hot Start Mix Red: The 2x mix contains VeriFi™ Hot Start Polymerase, 6 mM $MgCl_2$, 2 mM dNTPs, enhancers, stabilizers, and a red dye for tracking during agarose electrophoresis. It is not recommended to add further PCR enhancers or $MgCl_2$ to the reaction. The mix composition has been optimised to maximise PCR success rates.

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. 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.

Agarose gel electrophoresis dye migration: The 2x mix contains a red dye for tracking during agarose gel electrophoresis. In a 2% agarose TAE gel the dye migrates at a rate equivalent to 50-100 bp of DNA. In a 1% agarose TAE gel the dye migration rate is equivalent to 200-300 bp of DNA.

Reaction setup

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

Reagent	25 µL reaction	50µL reaction	Final concentration	Notes
2x VeriFi™ Hot Start Mix Red	12.5 µL	25.0 µL	1x	
Forward primer (10 µM)	1.0 µL	2.0 µL	400 nM	See above for optimal primer design
Reverse primer (10 µM)	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	
PCR grade dH_2O	Up to 25 µL final volume	Up to 50 µL final volume		

2. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	95 ˚C	1 min	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)