ELISABETH PHARMACON Ltd.
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EliZyme™ ProofRead HS

Intended use:

For Research Use Only. Not for use in diagnostic procedures.

Storage:

Upon arrival store components at -20 °C. Avoid prolonged exposure to light. When stored under these conditions, the kit will retain full activity until the expiration date indicated on the kit label. Reagents may be stored at 4 °C up to 1 month.

Product description

EliZyme™ ProofRead HS contains a polymerase derived from Pfu DNA polymerase, which has been modified with proprietary mutations to enhance its processivity, resulting in shorter extension times and improved yields. The enzyme is capable of amplifying longer and more difficult targets, including eukaryotic genomic templates larger than 17.5 kb. The innovative technology of EliZyme™ ProofRead HS employs a proprietary aptamer-like molecule that inhibits both the 3'-5' exonuclease activity and the 5'-3' polymerase activity of the enzyme at ambient temperatures. This unique hot start molecule prevents primer-dimer formation and unspecific amplification to increase the sensitivity and specificity of PCR reactions. The improved accuracy and enhanced 3'-5' exonuclease activity of EliZyme™ ProofRead HS polymerase result in a fidelity that is approximately 100 times higher than Taq DNA Polymerase (1 error per 2.5 x 10^7 nucleotides incorporated), making it ideal for high fidelity applications such as cloning, site-directed mutagenesis, and sequencing. The product line generates blunt-ended PCR products and includes an advanced buffer system with dNTPs, Mg, and enhancers, enabling high fidelity PCR of a wide range of targets and fragment sizes with minimal or no optimization. For added convenience, EliZyme™ ProofRead HS is available as a 2X Ready Mix w/out a red dye for tracking during agarose gel electrophoresis, suitable for direct loading onto agarose gels.

Content

	Ref. No.	Content	Size
EliZyme™ ProofRead HS	EZ0601	1×0.05 ml 2 U/µl + 1×1.7 ml buffer + 1×1.7 ml 10× Enhancer	100 U
	EZ0605	1×0.25 ml 2 U/µl + 3×1.7 ml buffer + 2×1.7 ml 10× Enhancer	500 U
	EZ0610	2×0.25 ml 2 U/µl + 6×1.7 ml buffer + 4×1.7 ml 10× Enhancer	1000 U
EliZyme™ ProofRead HS MIX	EZ0708	2×1 ml mix	80 rxns
	EZ0716	4×1 ml mix	160 rxns
	EZ0706	2×7.5 ml mix	600 rxns

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EliZyme™ ProofRead HS MIX Red	EZ0808	2×1 ml mix	80 rxns
	EZ0816	4×1 ml mix	160 rxns
	EZ0806	2×7.5 ml mix	600 rxns

	Buffer/MIX	Content
EliZyme™ ProofRead	5× buffer	15 mM $MgCl_2$, 5 mM dNTPs
EliZyme™ ProofRead MIX	2× mix	6 mM $MgCl_2$, 2 mM dNTPs
EliZyme™ ProofRead MIX Red	2× mix Red	6 mM $MgCl_2$, 2 mM dNTPs

Additional $MgCl_2$ is not necessary. The buffer composition has been optimized to maximize PCR success rates.

Primers

Primers should have a predicted melting temperature of around 60 °C. Primers should be designed to eliminate the possibility of primer-dimer formation and non-specific amplification. The final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

PCR

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. 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.

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 protocol may also be used with combined annealing and extension at 68–75 °C.

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.

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Reaction setup

EliZyme™ ProofRead HS

After thawing, briefly vortex 5X EliZyme™ PR HS Reaction Buffer and shortly spin.

Reagent	50 µl reaction	Final conc.
5X EliZyme™ PR HS Reaction Buffer	10 µl	1×
10× Enhancer (Optional)*	5 µl	1×
Forward primer (10 µM)	2 µl	400 nM
Reverse primer (10 µM)	2 µl	400 nM
Template DNA	< 200 ng genomic DNA, < 10 ng cDNA	Variable
EliZyme™ ProofRead HS Polymerase (2 U/µl)	0.5 µl	
PCR grade water	Up to 50 µl	

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

EliZyme™ ProofRead HS MIX

After thawing, briefly vortex the mix and shortly spin.

Reagent	50 µl reaction	Final conc.
2X EliZyme™ PR HS MIX	25 µl	1×
Forward primer (10 µM)	2 µl	400 nM
Reverse primer (10 µM)	2 µl	400 nM
Template DNA	<200 ng genomic DNA, < 10 ng cDNA	Variable
PCR grade water	Up to 50 µl	

EliZyme™ ProofRead HS MIX Red

After thawing, briefly vortex the mix and shortly spin.

Reagent	50 µl reaction	Final conc.
2X EliZyme™ PR HS MIX Red	25 µl	1×
Forward primer (10 µM)	2 µl	400 nM
Reverse primer (10 µM)	2 µl	400 nM
Template DNA	<200 ng genomic DNA, < 10 ng cDNA	Variable
PCR grade water	Up to 50 µl	

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PCR cycling profile

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	1 min	1
Denaturation	95 °C	15 s	25 - 35
Annealing	55 – 65 °C	15 s	
Extension	72 °C	10-30 s/kb*	

* see above for optimal extension time and multiplex considerations

Manufacturer:

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Catalog number

Batch code


Use by (last day of month)


Upper limit of temperature


Manufacturer

Contains sufficient "N" tests