ELISABETH PHARMACON Ltd.
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EliZyme™ ProofRead

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

The EliZyme™ ProofRead product line utilizes a polymerase derived from Pfu DNA polymerase with 3'-5' exonuclease activity for proofreading during PCR. The enzyme has undergone proprietary mutations that greatly enhance processivity, resulting in shorter extension times (10-30 s/kb), higher yields, and the ability to amplify longer and more challenging targets, including eukaryotic genomic templates larger than 17.5 kb. The fidelity of EliZyme™ ProofRead polymerase is about 100 times higher than that of Taq DNA polymerase (1 error per 2.5 x 10^7 nucleotides incorporated), making it an ideal choice for high-accuracy applications such as cloning, site-directed mutagenesis, and sequencing. The PCR products produced with this product line are blunt-ended. EliZyme™ ProofRead is supplied with a sophisticated buffer system containing dNTPs, Mg, and enhancers, which enables high-fidelity PCR of a wide variety of targets and fragment sizes with minimal or no optimization required. For even greater convenience, EliZyme™ ProofRead is also available as a 2X ready EliZyme™ ProofRead MIX, with EliZyme™ ProofRead MIX Red containing a red dye that allows for direct loading onto agarose gel after tracking during agarose gel electrophoresis.

Content

	Ref. No.	Content	Size
EliZyme™ ProofRead	EZ0301	1×0.05 ml 2 U/µl + 1×1.7 ml buffer + 1×1.7 ml 10× Enhancer	100 U
	EZ0305	1×0.25 ml 2 U/µl + 3×1.7 ml buffer + 2×1.7 ml 10× Enhancer	500 U
	EZ0310	2×0.25 ml 2 U/µl + 6×1.7 ml buffer + 4×1.7 ml 10× Enhancer	1000 U
EliZyme™ ProofRead MIX	EZ0408	2×1 ml mix	80 rxns
	EZ0416	4×1 ml mix	160 rxns
	EZ0406	2×7.5 ml mix	600 rxns
EliZyme™ ProofRead MIX Red	EZ0508	2×1 ml mix	80 rxns
	EZ0516	4×1 ml mix	160 rxns

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	EZ0506	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 protocols may also be used with combined annealing and extension at 68–75 °C.

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

EliZyme™ ProofRead

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

Reagent	50 µl reaction	Final conc.
5X EliZyme™ PR 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

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Template DNA	< 200 ng genomic DNA, < 10 ng cDNA	Variable
EliZyme™ PR 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 MIX

After thawing, briefly vortex the mix and shortly spin.

Reagent	50 µl reaction	Final conc.
2X EliZyme™ PR 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 MIX Red

After thawing, briefly vortex the mix and shortly spin.

Reagent	50 µl reaction	Final conc.
2X EliZyme™ PR 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	

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 fast cycling considerations

Manufacturer:

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Catalog number

Upper limit of temperature

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Batch code

Manufacturer

Use by (last day of month)

Contains sufficient "N" tests