ELISABETH PHARMACON Ltd.
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EliZyme™ HS FAST

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™ HS FAST uses hot-start technology to inactivate the enzyme below 65 °C preventing primer-dimer formation and non-specific amplification. The enzyme and buffer system allow for superior PCR performance on complex templates such as mammalian genomic DNA. Due to enhanced efficiency and specificity the enzyme is perfectly suited to difficult PCR. EliZyme™ HS FAST contains a robust enzyme ideal for all your everyday PCR applications including amplification of DNA for Sanger sequencing, genotyping, screening, library construction and multiplex PCR. The enzyme is particularly resistant to PCR inhibitors and is suitable for direct PCR from unprocessed samples including bacterial culture, bacterial colonies, blood and urine. EliZyme™ HS FAST has an error rate of approximately 1 error per 2.0 x 10^5 nucleotides incorporated. The enzyme system is characterized by enhanced PCR speed, yield and specificity. PCR products generated with EliZyme™ HS FAST are A-tailed and may be cloned into TA cloning vectors. EliZyme™ HS FAST delivers exceptional PCR performance on complex templates including GC-rich and AT-rich sequences. Polymerase included in EliZyme™ HS FAST has 5'-3' exonuclease activity but no 3'-5' exonuclease (proofreading) activity. DNA polymerase in EliZyme™ HS FAST is inactivated until the initial activation step at 95 °C. EliZyme™ HS FAST is suited for difficult PCR templates. The mix is resistant to PCR inhibitors allowing direct PCR from unprocessed samples including bacterial culture, bacterial colonies, blood and urine.

For higher comfort is EliZyme™ HS FAST also available in a 2X ready mix. EliZyme™ HS FAST MIX Red contains a red dye for tracking during agarose gel electrophoresis. It is suitable for direct loading onto agarose gel.

Content

	Ref. No.	Content	Size
EliZyme™ HS FAST	EZ5505	1×0.1 ml 5 U/µl + 4×1 ml buffer	500 U
	EZ5510	2×0.1 ml 5 U/µl + 1×8 ml buffer	1000 U
	EZ5520	4×0.1 ml 5 U/µl + 2×8 ml buffer	2000 U
EliZyme™ HS FAST MIX	EZ5720	5×1 ml mix	200 rxns

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	EZ5760	2×7.5 ml mix	600 rxns
EliZyme™ HS FAST MIX Red	EZ5620	5×1 ml mix	200 rxns
	EZ5660	2×7.5 ml mix	600 rxns

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

We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 55 °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. 20 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1 kb and 6 kb. For shorter amplicons, faster cycling is possible.

When first performing multiplex PCR it is recommended to run an annealing temperature gradient from 55 °C to 65 °C. The annealing temperature that results in the best specificity should be used in subsequent experiments. Fast cycling conditions should not be used for multiplex PCR. Initially, we recommend a 90 second extension time. This time may be further extended to increase yield.

From bacterial colonies use a sterile tip to pick a colony and resuspend into a 50 µl reaction as described below. From liquid culture add 5 µl of overnight culture to the final mix. Increase initial

denaturation time to 10 minutes. Add 2 µl mammalian blood or urine to a 50 µl reaction as described below.

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Reaction setup

EliZyme™ HS FAST

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

Reagent	50 µl reaction	Final conc.
5× EliZyme™ Reaction Buffer	10 µl	1×
Forward primer (10 µM)	2 µl	400 nM
Reverse primer (10 µM)	2 µl	400 nM
Template DNA	< 500 ng genomic DNA, < 100 ng cDNA	Variable
EliZyme™ HS Taq DNA Polymerase (5 U/µl)	0.25–1 µl	
PCR grade water	Up to 50 µl	

EliZyme™ HS FAST MIX

After thawing, briefly vortex the mix and shortly spin.

Reagent	50 µl reaction	Final conc.
2× EliZyme™ HS Taq MIX	25 µl	1×
Forward primer (10 µM)	2 µl	400 nM
Reverse primer (10 µM)	2 µl	400 nM
Template DNA	< 500 ng genomic DNA, < 100 ng cDNA	Variable
PCR grade water	Up to 50 µl	

EliZyme™ HS FAST MIX Red

After thawing, briefly vortex the mix and shortly spin.

Reagent	50 µl reaction	Final conc.
2× EliZyme™ HS Taq MIX Red	25 µl	1×
Forward primer (10 µM)	2 µl	400 nM
Reverse primer (10 µM)	2 µl	400 nM
Template DNA	< 500 ng genomic DNA, < 100 ng cDNA	Variable
PCR grade water	Up to 50 µl	

PCR cycling profile

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	1–2 min*	1
Denaturation	95 °C	15 s	40
Annealing	55–65 °C	15 s	
Extension	72 °C	1–90 s**	
Final extension	72 °C	90 s***	1

*For PCR from bacterial colonies extend initial denaturation time to 10 minutes.
**15 s/kb, for amplicons shorter than 1 kb ,1 second extension may be used
***Only for multiplex PCR.

Manufacturer:

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REF	Catalog number		Upper limit of temperature
LOT	Batch code		Manufacturer
	Use by (last day of month)	Σ	Contains sufficient "N" tests