Proteinase K(liquid for mNGS)

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Manual Version: V1.0

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(EC: 3.4.21.64, from *Tritirachium album*)

Proteinase K is a stable serine protease with broad substrate specificity. It degrades many proteins in the native state even in the presence of detergents. Evidence from crystal and molecular structure studies indicates the enzyme belongs to the subtilisin family with an active site catalytic triad (Asp^{39}-His^{69}-Ser^{224}). The predominant site of cleavage is the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups. It is commonly used for its broad specificity. This proteinase K is specially designed for mNGS. Compared with the other proteinase K, it contains even less nucleic acid contamination with the same enzymatic performance, which could better ensure the downstream mNGS application.

Product information

Product Name	Catalog	Size
Proteinase K (liquid)	HH4509-01	1 mL
	HH4509-02	5 mL
	HH4509-03	100 mL
	HH4509-04	1000 mL

Specification

Appearance	Colorless to light brown liquid
Activity	≥800 U/ml
Protein concentration	≥20 mg/ml
DNase	None detected
RNase	None detected
Nickase	None detected

Properties

EC number	3.4.21.64 (Recombinant from Tritirachium album)	
Molecular weight	29 kDa (SDS-PAGE)	
Isoelectric point	7.81	
Optimum pH	7.0-12.0	Fig. 1
Optimum temperature	65 °C	Fig. 2
pH stability	pH 4.5-12.5 (25 °C, 16 h)	Fig. 3
Thermal stability	Below 50 °C (pH 8.0, 30 min)	Fig. 4
Storage stability	Over 90% activity for 12months at 25°C	Fig. 5
Activators	SDS, urea	
Inhibitors	Diisopropyl fluorophosphate; phenylmethylsulfonyl fluoride	
Storage condition	2-8 °C	

Applications

Genetic diagnostic kit; RNA and DNA extraction kits; Extraction of non-protein components from tissues, degradation of protein impurities, such as DNA vaccines and preparation of heparin; Preparation of chromosome DNA by pulsed electrophoresis; Western blot; Enzymatic glycosylated albumin reagents in vitro diagnosis.

Precautions

Wear protective gloves and goggles when using or weighing, and keep well ventilated after use. This product may cause skin allergic reaction. Cause serious eye irritation. If inhaled, it may cause allergy or asthma symptoms or dyspnea. May cause respiratory irritation.

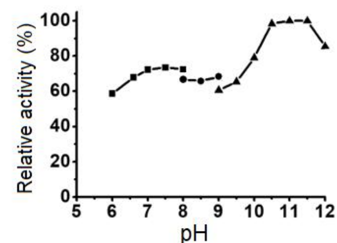

Fig. 1 Optimum pH

100 mM buffer solution: pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-12.5, Glycine-NaOH. Enzyme concentration: 1 mg/mL

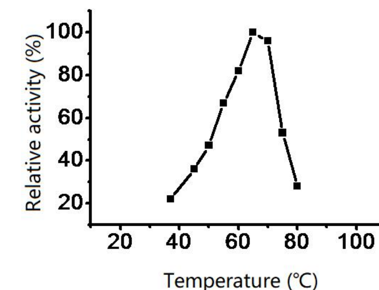

Fig. 2 Optimum temperature

Reaction in 20 mM K-phosphate buffer pH 8.0. Enzyme concentration: 1 mg/mL

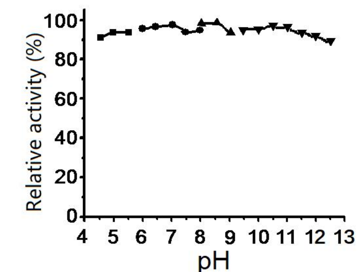

Fig. 3 pH Stability

25 ℃, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, Acetate; pH 6.0-8.0, Na- phosphate; pH 8.0-9.0, Tris-HCl. pH 9.0-12.5, Glycine-NaOH. Enzyme concentration: 1 mg/mL

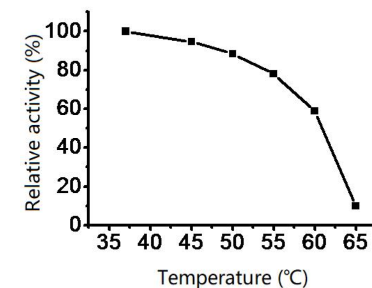

Fig. 4 Thermal stability

30 min-treatment with 50 mM Tris-HCl buffer, pH 8.0.
Enzyme concentration: 1 mg/mL

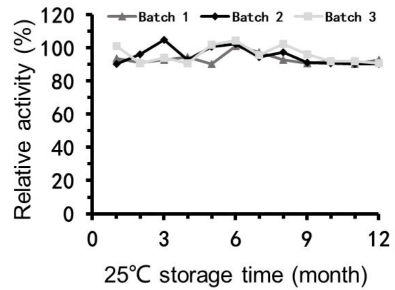

Fig. 5 Storage stability at 25℃

Unit definition

One unit (U) is defined as the amount of enzyme required to hydrolyze casein to produce 1 μmol tyrosine per minute under the following conditions.

Reagents preparation

Reagent I: 1 g milk casein was dissolved in 50 mL of 0.1 M sodium phosphate solution (pH 8.0), incubated in 65-70 ℃ water for 15 min, stirred and dissolved, cooled by water, adjusted by sodium hydroxide to pH 8.0, and fixed volume 100mL.

Reagent II: TCA solution: 0.1 M trichloroacetic acid, 0.2 M sodium acetate, 0.3 M acetic acid.

Reagent III: 0.4 M Na2CO3 solution.

Reagent IV: Forint reagent diluted with pure water for 5 times.

Reagent V: enzyme diluent: 0.1 M sodium phosphate solution (pH 8.0).

Reagent VI: tyrosine solution: 0, 0.005, 0.025, 0.05, 0.075, 0.1, 0.25 umol/mL tyrosine dissolved with 0.2 M HCl.

Procedure

0.5 mL of reagent I is pre-warmed to 37℃, add 0.5 mL of enzyme solution, mix well, and incubate at 37℃ for 10 min.

Add 1 mL of reagent II to stop the reaction, mix well, and continue incubation for 30 min.

Centrifugate reaction solution.

Take 0.5 mL supernatant, add 2.5 mL reagent III, 0.5 mL reagent IV, mix well and incubate at 37℃ for 30 min.

OD660 was determined as OD1; blank control group: 0.5 mL reagent V is used to replace enzyme solution to determine OD_{660} as OD_2, $\triangle OD=OD_1-OD_2$.

L-tyrosine standard curve: 0.5mL different concentration L-tyrosine solution, 2.5mL Reagent III, 0.5mL Reagent IV in 5mL centrifuge tube, incubate in 37℃ for 30min, detect for OD660 for different concentration of L-tyrosine, then obtained the standard curve Y=kX+b, where Y is the L-tyrosine concentration, X is OD_{660}.

Calculation

$$\textbf{Volume activity } (U/ml) = \frac{(k\triangle \text{OD}+\text{b})\times \text{df}\times 2}{10\times 0.5\times 0.5}$$

2: Total volume of reaction solution (mL)

0.5: Volume of enzyme solution (mL)

0.5: Reaction liquid volume used in chromogenic determination (mL)

10: Reaction time (min)

Df: Dilution multiple

C: Enzyme concentration (mg/mL)

References

1. Wieger U & Hilz H. FEBS Lett. (1972); 23:77.

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