Proteinase K (lyophilized powder for NGS)

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Manual Version: V1.1

Product description

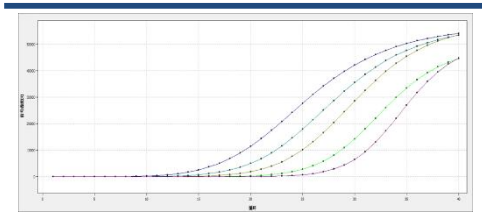
NGS Protease K is a serine protease with high enzyme activity and wide substrate specificity. The enzyme preferentially decomposes ester bonds and peptide bonds adjacent to the C-terminal of hydrophobic amino acids, sulfur-containing amino acids and aromatic amino acids. So, it is often used to degrade proteins into short peptides. NGS Protease K is a typical serine protease with the Asp39-His69-Ser224 catalytic triad which is unique to serine proteases, and the catalytic center is surrounded by tow Ca^{2+} binding sites for stabilization, maintaining high enzyme activity under a wider range of conditions.

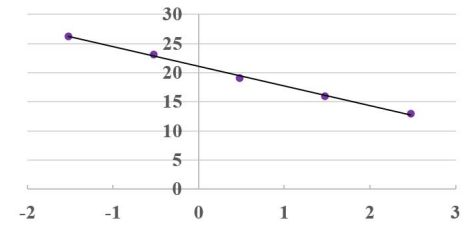
Product information

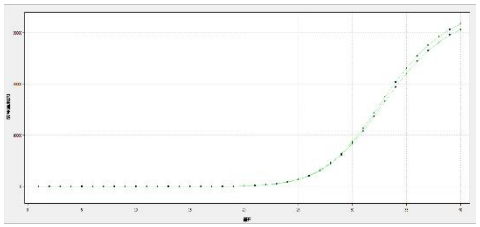
Product Name	Catalog	Size
Proteinase K (lyophilized powder for NGS)	HH4507-01	100 mg
	HH4507-02	1 g
	HH4507-03	10 g
	HH4507-04	100 g

Specification

	lyophilized
Specific activity	≥40 U/mg solid
DNase	None detected
RNase	None detected
Nickase	None detected
Bioburden	≤50CFU/g solid
Nucleic acid residue	<5pg/mg solid







Sample	Ave C_t	Nucleic Acid Recovery (pg/mg)	Recovery (%)	Total Nucleic Acid (pg/mg)
PRK	24.66	2.23	83%	2.687
PRK+STD2	18.723	126.728	——	——
STD1	12.955	——	——	——
STD2	16			
STD3	19.125			
STD4	23.135			
STD5	26.265			
RNA-Free H2O	Undetermined	——	——	——

Fig. 1 DNA residue

Properties

Source	Tritirachium album	
classification	EC 3.4.21.64	
Molecular weight	29 kDa (SDS-PAGE)	
Isoelectric point	7.81	
Optimum pH	7.0-12.0 (All perform high activity)	Fig. 2
Optimum temperature	65 ℃	Fig. 3
pH Stability	pH 4.5-12.5 (25 ℃, 16 h)	Fig. 4
Thermal stability	Below 50℃ (pH 8.0, 30 min)	Fig. 5
Storage stability	Stored at 25℃ for 12months	Fig. 6
Activator	SDS, Urea	
Inhibitor	diisopropylfluorophosphate (DFIP), benzylsulfonyl fluoride (PMSF)	

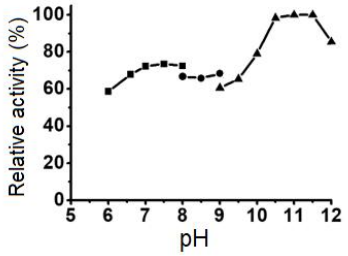


Fig. 2 Optimum pH

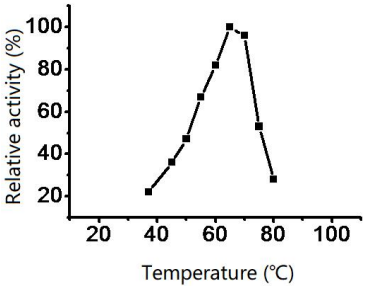


Fig. 3 Optimum temperature

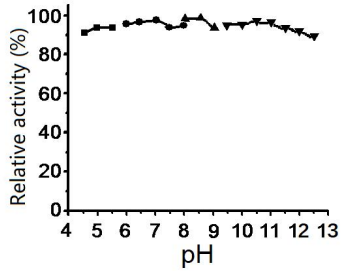


Fig. 4 pH Stability

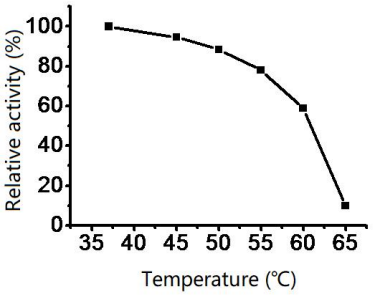


Fig. 5 Thermal stability

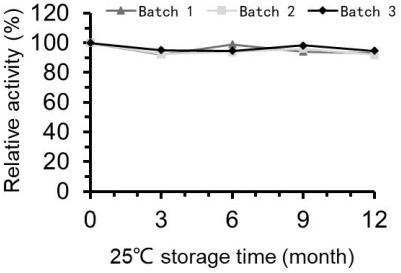


Fig. 6 Storage stability at 25℃

Transportation & preservation

Store the lyophilized powder at -25 ~ -15 ℃ for a long time away from light ; After dissolution, aliquot into appropriate volume for short-term storage at 2-8 ℃ away from light or long-term storage at -25 ~ -15 ℃ away from light.

Unit definition

One unit of NGS Protease K is defined as the amount of enzyme required to hydrolyze casein into 1 μmol L-tyrosine under standard determination conditions.

Reagents preparation

Reagent	manufacturer	catalog
Casein technical from bovine milk	Sigma Aldrich	C7078
NaOH	Sinopharm Chemical Reagent Co.,Ltd.	10019762
$NaH_2PO_4 \cdot 2H_2O$	Sinopharm Chemical Reagent Co.,Ltd.	20040718
Na_2HPO_4	Sinopharm Chemical Reagent Co.,Ltd.	20040618
Trichloroacetic acid	Sinopharm Chemical Reagent Co.,Ltd.	80132618
Sodium acetate	Sinopharm Chemical Reagent Co.,Ltd.	10018818
acetic acid	Sinopharm Chemical Reagent Co.,Ltd.	10000218
HCl	Sinopharm Chemical Reagent Co.,Ltd.	10011018
Sodium carbonate	Sinopharm Chemical Reagent Co.,Ltd.	10019260
Foline-phenol	Sangon Biotech (Shanghai) Co., Ltd.	A500467-0100
L-tyrosine	Sigma	93829

Reagent I

Substrate:

1% Casein from bovine milk solution: dissolve 1g bovine milk casein in 50 ml of 0.1 M sodium phosphate solution, pH 8.0, heat in water bath at 65-70 ℃ for 15 min, stir and dissolve, cool with water, adjusted by sodium hydroxide to pH 8.0, and dilute in to 100 ml.

Reagent II:

TCA solution：

0.1M trichloroacetic acid, 0.2m sodium acetate and 0.3m acetic acid (weigh 1.64g trichloroacetic acid + 1.64g sodium acetate + 1.724ml acetic acid successively, add 50mL deionized water, adjust with HCl to pH 4.03, and dilute into 100ml).

Reagent III:

0.4m sodium carbonate solution (weigh 4.24g anhydrous sodium carbonate and dissolve in 100ml water).

Reagent IV:

Folin phenol reagent: dilute 5 times with deionized water.

Reagent V:

Enzyme diluent: 0.1 M sodium phosphate solution, pH 8.0.

Reagent VI:

L-tyrosine standard solution: 0, 0.005, 0.025, 0.05, 0.075, 0.1, 0.25 μmol/ml L-tyrosine dissolved with 0.2 M HCl.

Procedure

a. Turn on the UV-Vis spectrophotometer and select photometric measurement.
b. Set the wavelength as 660nm.
c. Turn on the water bath, set the temperature to 37 ℃, ensure the temperature unchanged for 3-5min.
d. Preheat 0.5ml substrate in a 2mL centrifuge tube at 37 ℃ water bath for 10min.
e. Extract 0.5mL diluted enzyme solution into the preheated centrifuge tube for 10 minutes. Set enzyme diluent as blank group.
f. Add 1.0 mL TCA reagent immediately after the reaction. Mix well and incubate in water bath for 30 minutes.
g. Centrifugate reaction solution.
h. Add the following components in the order specified.

Reagent	Volume
Supernatant	0.5 mL
0.4M Sodium carbonate	2.5 mL
Folin phenol reagent	0.5 mL

i. mix well before incubating in water bath a 37 ℃ for 30 min.
j. OD_{660} was determined as OD_1; blank control group: Enzyme diluent is used to replace enzyme solution to determine OD_{660} as OD_2, $\triangle OD=OD_1-OD_2$.
k. L-tyrosine standard curve: 0.5mL different concentration L-tyrosine solution, 2.5mL 0.4M Sodium carbonate, 0.5mL Folin phenol reagent in 5mL centrifuge tube, incubate in 37℃ for 30min, detect for OD_{660} for different concentration of L-tyrosine, then obtained the standard curve Y=kX+b, where Y is the L-tyrosine concentration, X is OD_{600}.

Calculation

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

$$\text{Weight activity}(U/mg) = \text{Volume activity} \times 1/C$$

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)