ELK Biotechnology

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Proteinase K

Catalog No.	Specification	Storage/Shelf life
EQ023	1mL	-20℃/2 year

Introductoin

Proteinase K is a serine protease that belongs to the subtilisin family. It has extremely high enzyme activity and wide substrate specificity. It can preferentially decompose the ester bonds and peptide bonds adjacent to the C-terminus of hydrophobic amino acids, sulfur-containing amino acids, and aromatic amino acids. It is often used to degrade proteins to produce short peptides. It has the typical catalytic triad Asp39-His69-Ser224 characteristics unique to serine proteases, and there are two Ca2+ binding sites around the active center to increase its stability, allowing it to maintain high enzyme activity under more extensive conditions.

Enzymatic properties

Source:	Limbert Candida albicans
Classification	EC 3.4.21.64
Molecular weight	29 kDa (SDS-PAGE)
Isoelectric point	7.81
Optimal pH	7.0-12.0 All have high activity
Optimum temperature	65 ℃
pH stability	pH 4.5-12.5 (25 ℃, 16 h)
Thermal stability	Under 50 ℃ (pH 8.0, 30min)
Activator	SDS, Urea
Inhibitor	DFIP,PMSF
Storage conditions	Drypowder state can be stored at-20 ℃for a long time； after dissolution,it should be divided into appropriate volume， short-term storage at 2-8 ℃， long-term storage at-20 ℃

Definition method of activity determination

The unit enzyme activity is defined as the amount of enzyme required to hydrolyze

casein to produce 1umol of tyrosine per minute under the following conditions.

Application

1.Genetic diagnosis kit；2.RNA and DNA extraction kit；3.Extract non-protein components from tissues and degrade protein impurities,such as preparation of DNA vaccines and heparin；4.Preparation of chromosomal DNA for pulse electrophoresis；5.Western Blot；6.For the development and mass production of enzymatic glycated albumin reagents ine the field of in vitro diagnostics.

Reagent preparation

Reagent I：Substrate：1%milk casein solution：1 g milk casein dissolved in 50 ml 0.1 M sodium phosphate solution， pH 8.0，incubate in a 65-70 ℃ water bath for 15 minstir to dissolve,cool in tap water,adjust with sodium hydroxide pH 8.0，constant volume 100 ml.

Reagent II： TCA solution： 0.1 M trichloroacetic acid，0.2 M sodium acetate，0.3 M acetic acid，HCl adjust pH 4.03，volume 100ml.

Reagent III：0.4 M Sodium carbonate solution.

Reagent IV：Folin reagent：Dilute 5 times with water.

Reagent V：Enzyme diluent：0.1 M Sodium phosphate solution，pH 8.0.

Reagent VI：Tyrosine solution：1 μg/ml Tyrosine，0.2 M HCI dissolve.

Steps

1．Incubate 0.5 ml reagent I at 37 ℃ for 10 min，add 0.5 ml enzyme solution，mix well，and react at 37 ℃ for 10 min；

2. Add 1 ml Reagent II to stop the reaction,mix well，and incubate for 30 min；

3. Centrifuge reaction solution；

4.Take 0.5 ml supernatant and add 2.5 ml Reagent III，

0.5 ml Reagent IV，mix well and incubate 30min at 37 ℃；

5．660 nm measure OD1；blank control group：0.5 ml Reagent V instead of enzyme solution，measure OD2；

6．0.5 ml Reagent VI，2.5 ml Reagent III，0.5 ml Reagent IV，mix well and incubate 30min at 37 ℃.660 nm measure OD3；blank control group：0.5 ml 0.2 M HCI instead of Reagent VI，measured value is OD4。

Vitality calculation

Volume activity (U/mL)

$$=\frac{(OD1-OD2) \times df \times 2}{(OD3-OD4)\times 10 \times 181.2 \times 0.5}$$

Weight activity(U/mg) =Volume activity×1/C

2：Total volume of reaction solution（mL）；

0.5：Enzyme liquid volume（mL）；

10：Reaction time（min）； df： Dilution times ； 181.2：Tyrosine molecular weight；

C：Enzyme concentration(mg/mL)。