Proteinase K

Proteinase K from the fungus *Tritirachium album* is a nonspecific serine protease that is useful for general digestion of proteins. Removal of endogenous nucleases during the preparation of DNA and RNA; preparation of tissue sections for *in situ* hybridization.

Technical Data

Appearance	White amorphous lyophilized powder
Protein purity	≥95%
Specific activity	≥30 U/mg Protein
Deoxyribonuclease activity	No activity of deoxyribonuclease was detected
Ribonuclease activity	No activity of Ribonuclease was detected

Enzymatic Properties

Source	*Tritirachium album*	
Classification	EC 3.4.21.64	
Molecular weight	29 kDa (SDS-PAGE)	
Isoelectric point	7.81	
Optimal pH	7.0-12.0 Both have high activity	Char1
Optimum temperature	65 ℃	Chart2
pH stability	pH 4.5-12.5 (25 ℃, 16 h)	Chart3
Thermal stability	Stable below 50 ℃（pH 8.0, 30 min）	Chart4
Activator	SDS, Urea	
Inhibitor	Diisopropylfluorophosphate (DFIP)，Benzenesulfonyl fluoride (PMSF)	
Storage conditions	Dry powder can be stored at - 20 ℃ for a long time; After dissolving, it should be packed into proper volume, stored at 2-8 ℃ for a short time, and stored at - 20 ℃ for a long time.	

Use:

The virus consists of a protein shell and an internal nucleic acid. To amplify the nucleic acid by PCR, the shell protein must be destroyed first. The role of protease K is to break down the protein shell of the virus, releasing nucleic acid for easy detection.

1. Gene diagnosis kit；2. RNA and DNA Extraction Kit；3. Extract non protein components from tissues and degrade protein impurities. For example, the preparation of DNA vaccine and heparin；4.

Preparation of chromosome DNA by pulse electrophoresis；5. Western blotting；6. R&D and mass production of enzyme based glycated albumin reagents for in vitro diagnosis.

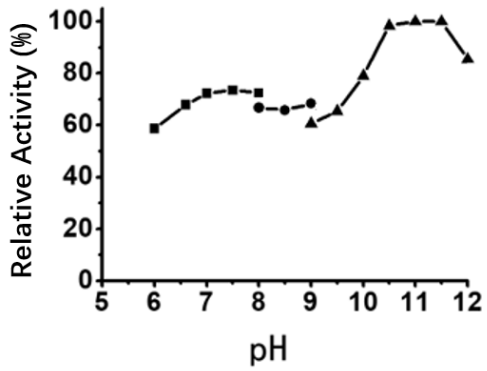

Chart 1 Optimal pH

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.

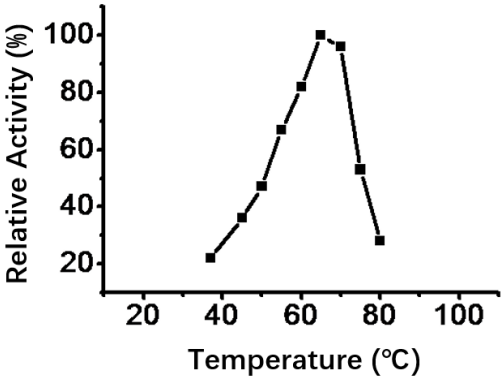

Chart 2 Optimum temperature

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

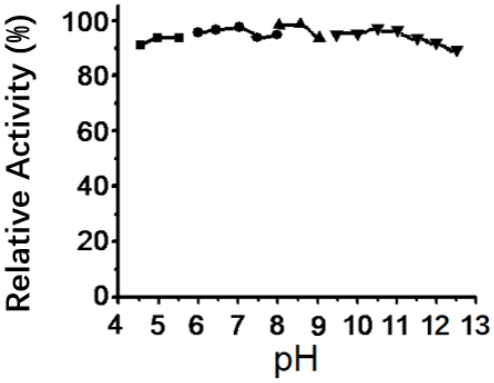

Chart 3 pH stability

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 9.5-12.5, Glycine-NaOH.
Enzyme concentration: 1 mg/mL.

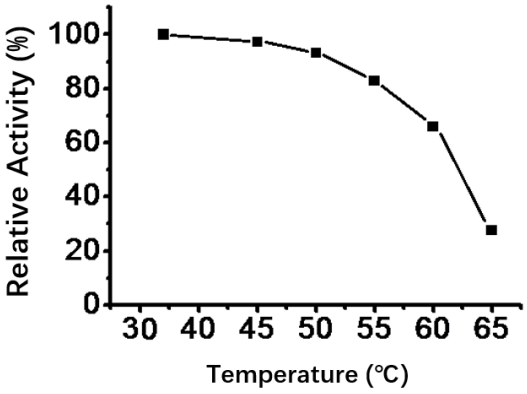

Chart 4 Thermal stability

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

Activity determination method

Enzyme activity definition

Unit enzyme activity is defined as the amount of enzyme required to hydrolyze casein to produce 1 μ mol tyrosine per minute under the following conditions.

Reagent preparation

Reagent I： Substrate：1% milk casein solution. 1g of 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 tap water, adjusted pH 8.0 by sodium hydroxide, and fixed volume of 100 ml.

Reagent II： TCA Solution：0.1 M Trichloroacetic acid，0.2 M Sodium acetate，0.3 M Acetic acid , HCl regulates pH 4.03 and constant volume 100ml.

Reagent III：0.4 M Sodium carbonate solution

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

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 Solution.

Operation steps

1．0.5 ml of reagent I was incubated at 37 ℃ for 10 min, added with 0.5 ml of enzyme solution, mixed well, and reacted at 37 ℃ for 10 min；

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

3．Centrifugal reaction liquid；

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

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

6．0.5 ml of reagent VI, 2.5 ml of reagent III and 0.5 ml of reagent IV were mixed and incubated at 37 ℃ for 30 min. Od3 was measured at 660 nm; in the blank control group, 0.5 ml 0.2 M HCI was used instead of reagent VI, and the measured value was OD4.

Activity Calculation

$$\text{Volume activity}\,(U/ml) = \frac{(\text{OD1}-\text{OD2})\times \text{df}\times 2}{(\text{OD3}-\text{OD4})\times 10\times 181.2\times 0.5}$$

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

2：Total volume of reaction liquid（mL）；

0.5：Enzyme volume（mL）；

10：Reaction time（min）；

df：Dilution ratio；

181.2：Molecular weight of tyrosine；

C：Enzyme concentration (mg/mL)。