

# PROTEINASE K – FREQUENTLY ASKED QUESTIONS

## QUESTION

## ANSWER

**What are the specific properties of Proteinase K?**

Proteinase K is a non-specific serine protease with a very high specific activity. It is active in the presence and absence of SDS and EDTA. The enzyme is used for

- (1) Digestion of unwanted proteins in molecular biology applications;
- (2) Removal of endotoxins bound to cationic proteins such as lysozyme and RNase A;
- (3) Removal of nucleases for in situ hybridization;
- (4) Prion research with respect to TSE (Transmissible Spongiform Encephalopathies);
- (5) Protease footprinting;
- (6) Mitochondrial isolation;
- (7) Isolation of genomic DNA;
- (8) Isolation of cytoplasmic RNA;
- (9) Isolation of highly native DNA or RNA

**How do you inactivate Proteinase K?**

Proteinase K is inactivated by heating at 95°C for 10 minutes. However, heating of proteinase K does not fully inactivate the enzyme because there will always be a small amount of activity remaining. - Protease inhibitors such as PMSF and AEBSF can be used to permanently inactivate proteinase K.

**What is the optimum temperature for Proteinase K activation?**

Proteinase K activity increases with temperature, up to the optimal temperature range between 50-65°C. Higher temperatures help with protein unfolding, easing the ability for proteinase K to breakdown those proteins.

**What exactly is the relationship between Proteinase K and calcium?**

Proteinase K binds to two Ca<sup>2+</sup> ions which help maintain the stability of the enzyme, especially when it is subjected to increasing temperatures. Calcium also protects proteinase K from autolysis. While calcium helps maintain proteinase K thermostability, it is not necessary for proteolytic activity.

**What about Proteinase K activity in molecular biological application?**

According to Richard Tullis and Harvey Rubin, this relationship becomes more interesting when DNase I is involved. Proteinase K is known to inactivate DNases and RNases. In the presence of Ca<sup>2+</sup>, DNase I is protected from Proteinase K (concentration of 1mg/ml). RNase, however, is inactivated in the presence of Ca<sup>2+</sup>. These properties suggest a method for treating contaminated RNase free DNase I or isolating highly polymerized RNA.

**Does EDTA inactivate Proteinase K?**

This question also seems to come up quite often in discussions about Proteinase K. Chelators such as EDTA or EGTA do not have a direct effect on proteinase K enzyme activity. Use EDTA together with proteinase K is for the removal of calcium during DNA or RNA purification.

**What are the activators of Proteinase K?**

Proteinase K activators include SDS (Sodium Dodecyl Sulfate) and Urea.

**How is Proteinase K involved with cell lysis?**

Proteinase K is a broad-spectrum protease capable of digesting a wide range of native proteins. When it comes to cell lysis, particularly for downstream DNA isolation and purification, Proteinase K can be part of the lysis step by digesting surface proteins. During further procedure steps of resuspension and lysis of nuclei, the preparation buffer contains proteinase K supporting digest of proteins that might degrade the nucleic acid sample.

**Why do many DNA extraction lysis buffer recipes call for Proteinase K and RNase?**

Proteinase K inactivates nucleases during DNA/RNA extraction procedures. This occurs because Proteinase K is capable of digesting those proteins that might degrade the sample.

**Why is knowledge about activity of Proteinase K important? How is the activity defined?**

Enzyme activity is the number of moles or mg, etc. of substrate modified by an enzyme in a given time frame (e.g. µmol/min). Specific activity is related to enzyme purity. It is the amount of substrate modified by a particular quantity of protein in an enzyme preparation per unit of time.  
Enzyme Concentration: Your enzyme concentration is simply the number of units of activity per volume.

**How do you prepare a Proteinase K stock solution?**

By protocol for preparing a 20 mg/ml stock solution, use Tris buffer and CaCl<sub>2</sub>.

**What is the shelf-life of Proteinase K?**

Stock Solution: aliquot your stock solution and store at -20°C for up to 1 year.  
Lyophilized Powder: Store desiccated at -20°C for up to 2 years.

General

Specialities

Specific applications



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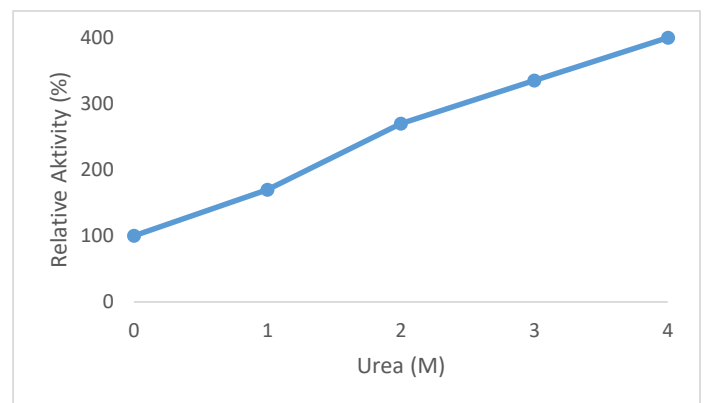
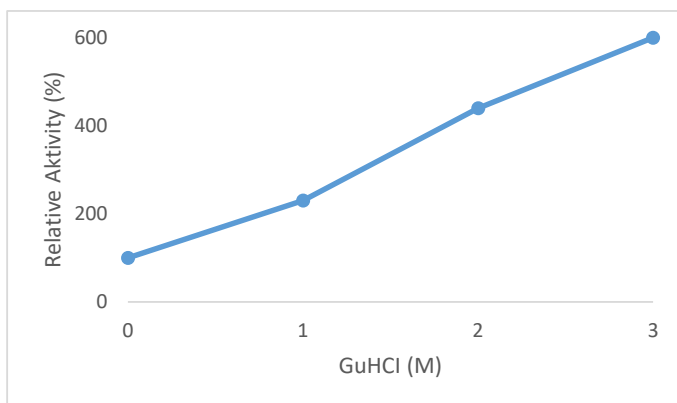
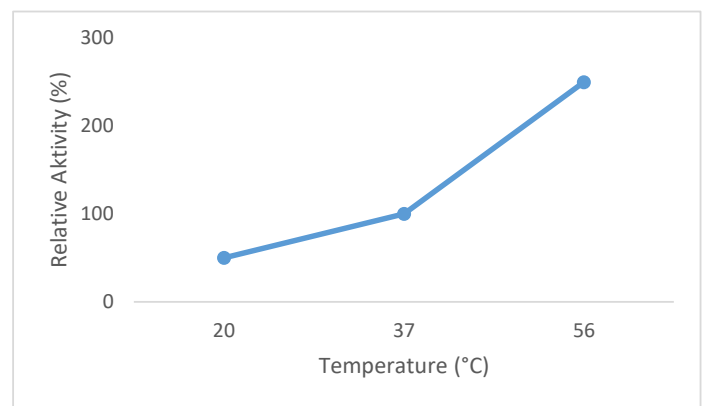
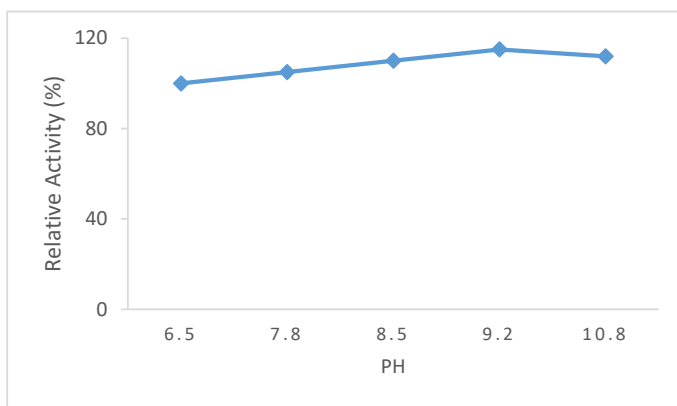
**What is difference between native and recombinant Proteinase K?**

Native Proteinase K is a broad-spectrum serine protease extracted from the fungus *Engyodontium album* (formerly *Tritirachium album* Limber). The enzyme belongs to the subtilisin family consisting of 279 amino acids with its active-site catalytic triad of Asp39-His69-Ser224. It is stable in a broad range of environments: pH, buffer salts, detergents (e.g. SDS), and temperature. Heating Proteinase K to 95°C for 10 minutes will inactivate it. The  $K_m$  value (indicating the affinity of an enzyme for a specific substrate) of recombinant Proteinase K is nearly identical with the  $K_m$  value of native Proteinase K. Fast-propagating yeast host cells of the species *Pichia*, *Hansenula*, *Saccharomyces*, and *Schizosaccharomyces* can be used for high-yield production. Thus, Larger amounts of the recombinant proteinase K of higher purity and in a soluble form can be obtained. This has almost the same properties as the native form by the original fungal cells with a certain slower propagation rate. However, the recombinant enzyme is rapidly denatured at temperatures above +65°C. Therefore, the shelflife of the recombinant form is lower than for the native one.

**How is the activity of Proteinase K under various buffer conditions?**

See graphic below

Specific applications



Reference: Personal correspondence 1997

Buffer	Proteinase K Activity (%)
30 mM Tris	100
30 mM Tris-HCl; 30 mM EDTA; 5% Tween 20, 0,5% Tergitol™; 800 mM GuHCl	313
36 mM Tris-HCl; 36 mM EDTA; 5% Tween 20; 0,36% Tergitol™; GuHCl	301
10 mM Tris-HCl; 25 mM EDTA; 1200 mM NaCl; 0,5% SDS	128
10 mM Tris-HCl; 100 mM EDTA; 20 mM NaCl; 1% Sarkosyl	74
10 mM Tris-HCl; 50 mM KCl; 15 mM MgCl, 0,45% Tween 20; Tergitol™	106
10 mM Tris-HCl; 100 mM EDTA; 0,5% SDS	120
30 mM Tris-HCl; 10 mM EDTA; 1% SDS	203

