Product Information

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Proteinase K
from *Engyodontium album*
(formerly *Tritirachium album*)

Catalog Number **P6556**
Storage Temperature –20 °C

CAS RN 39450-01-6
E.C. 3.4.21.64
Synonyms: Peptidase K, Endoproteinase K, Endopeptidase K

Product Description

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. Proteinase K was isolated from a fungus able to grow on keratin and the enzyme can digest native keratin (hair); hence its name "Proteinase K".[1] 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.[2-4] The mode and specificity of action has been studied.[4]

Proteinase K is frequently used in molecular biology applications to digest unwanted proteins, such as nucleases from DNA or RNA preparations from microorganisms, cultured cells, and plants.[5-11] The enzyme is typically used at 50–200 μg/ml in nucleic acid preparations at pH 7.5–8.0 and 37 °C. Incubation times vary from 30 minutes to 18 hours. Proteinase K is usually denatured by subsequent phenol extractions, although it can autodigest during long incubations.[3] Catalog Numbers P2308 and P4850 (a solution in 40% (v/v) glycerol and Tris buffer) are tested for suitability in molecular biology applications.

Proteinase K has been used to remove endotoxins bound to cationic proteins such as lysozyme and ribonuclease A.[12] It has been used for determination of enzyme localization on membranes,[13] treatment of paraffin embedded tissue sections to expose antigen binding sites for antibody labeling,[14] and remove nucleases for *in situ* hybridization.[15] Research on prions in Transmissible Spongiform Encephalopathies (TSE) and proposed diagnostic tests utilize Proteinase K digestion of proteins from brain tissue samples.[16,17] Protease footprinting by Proteinase K digestion can reveal protein-protein surface interactions.[18]

Proteinase K is active in 1% Triton™ X-100 and fully active in 0.5% (w/v) SDS. SDS and urea will denature protein substrates resulting in increased digestion rates. Proteinase K itself is denatured much more slowly by these agents.[3,19,20]

Molecular mass: 28,930 Da (amino acid sequence)[21]
28,500 Da (SDS-PAGE)[22]

pH range:[2,3] 7.5–12.0 (urea-denatured hemoglobin as substrate), but most often used in pH range 7.5–9.0.

Temperature profile:[3] maximum activity at 37 °C
(In the temperature range of 20–60 °C, a minimum of 80% of the activity observed at 37 °C is expected)

pI:[2] 8.9

Extinction coefficient:[2] $E^{1\%}$ = 14.2 (280 nm, 10 mM NaCl and 5 mM $CaCl_2$, pH 8.0)

Activators: 1–5 mM Ca^{2+} is required for activation. When calcium is removed from the enzyme (by addition of EDTA), 25% of the catalytic activity is lost. However, if the EDTA-Ca^{2+} complex is removed from the enzyme solution by gel filtration, a total of 80% of the enzyme activity is lost and only a small activation will occur upon addition of excess Ca^{2+} to the Ca^{2+}-free enzyme.[23]

Inhibitors: Proteinase K is inhibited by DIFP or PMSF (the latter used at final concentration 5 mM).[3] It is partly inactivated, but not inhibited, by EDTA (see Activators). Proteinase K is not inhibited by iodoacetic acid, the trypsin-specific inhibitor TLCK, the chymotrypsin-specific inhibitor TPCK, and *p*-chloromercuribenzoate.

This product is supplied as a lyophilized powder.

Specific activity: ≥30 units/mg of protein

Unit Definition: One unit will hydrolyze urea-denatured hemoglobin to produce color equivalent to 1.0 μmole (181 μg) of tyrosine per minute at pH 7.5 at 37 °C.

Enzymatic Impurities:
DNase: ≤30 units/mg solid
RNase: ≤0.003 units/mg solid

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
This product is soluble in water (1 mg/ml), yielding a clear colorless solution.

Storage/Stability
Sigma recommends storage at –20 °C, though others have claimed stability at 2–8 °C. When stored at –20 °C, the product retains activity for at least 2 years.

A Proteinase K solution remains active over a broad pH range (4.0–12.5, optimum pH 8.0) and also over the temperature range of 25–65 °C during use. At pH 8.0, solutions remain active for at least 12 months at 2–8 °C.[3] At pH 4–11.5, solutions containing Ca^{2+} (1–6 mM) are expected to remain active for several weeks. An 80% ammonium sulfate suspension stored at 2–8 °C remains active for at least 12 months.[2]

References

1. Betzel, C., *et al.*, *Eur. J. Biochem.*, **178(1)**, 155-171 (1988).
2. Ebeling, W., *et al*., *Eur. J. Biochem.,* **47(1)**, 91-97 (1974).
3. *Enzymes of Molecular Biology*, Vol. **16** (Burrell, M.M., ed.),. Humana Press (Totowa, NJ), p. 307 (1993).
4. Kraus, E., and Femfert, U., *Hoppe Seylers Z. Physiol. Chem.,* **357(7)**, 937-947 (1976).
5. Lizardi, P.M., and Engelberg, A., *Anal. Biochem.,* **98(1)**, 116-122 (1979).
6. Gross-Bellard, M., *et al*., *Eur. J. Biochem.,* **36(1)**, 32-38 (1973).
7. *Molecular Cloning: A Laboratory Handbook*, 2nd ed. (Sambrook, J., *et al*., eds.), Cold Spring Harbor Press (Cold Spring Harbor, NY) p. 1.61 and p. B.16 (1989).
8. Kasche, V., *et al*., *Prep. Biochem*., **11(3)**, 233-250 (1981).
9. Hansen, J.N., *Prep. Biochem*., **4(6),** 473-488 (1974).
10. Holm, C., *et al*., *Gene*, **42(2)**, 169-173 (1986).
11. La Claire, J.W., and Herrin, D.L., *Plant Mol. Biol. Reporter*, **15(3)**, 263-272 (1997).
12. Petsch, P., *et al*., *Anal. Biochem*., **259(1)**, 42-47 (1998).
13. Brdiczyka, D., and Krebs, W., *Biochem. Biophys. Acta*, **297(2)**, 203-212 (1973).
14. Short, B.G., *et al*., *J. Histochem. Cytochem.*, **45(9)**, 1299-1305 (1997).
15. Angerer, L.M., *et al*., *Methods Enzymol.,* **152**, 649-661 (1987).
16. Sakaguchi, S., *et al*., *J. Virology*, **69(12)**, 7586-7592 (1995).
17. Bennion, B.J., and Daggett, V., *Clin. Chem.*, **48(12)**, 2105-2114 (2002).
18. Hori, R., and Carey, M., *J. Biol. Chem*., **272(2)**, 1180-1187 (1997).
19. Hilz, H., *et al*., *Eur. J. Biochem.*, **56(1)**, 103-108 (1975).
20. *Methods of Enzymatic Analysis*, 3rd Edition (Bergmeyer, H.U., ed.), Academic Press (New York, NY), Vol. **2**, p. 299 (1983).
21. Jany, K.-D., *et al*., *FEBS Lett.*, **199(2)**, 139-144 (1986).
22. Jany, K.-D., and Mayer, B., *Biol. Chem. Hoppe-Seyler*, **366(5)**, 485-492 (1985).
23. Bajorath, J., *et al*., *Eur. J. Biochem*., **176(2)**, 441-447 (1988).

Triton is a trademark of The Dow Chemical Company or an affiliated company of Dow.

RBR,RBG,GCY,MAM 01/16-1