

Proteinase K from *Tritirachium album* Cat. No. 33752

Product Description:

General Proteinase K¹ is a non-specific serine protease with a very broad range of action. It has been shown to exhibit a high degree of sequence homology with the subtilisin family of proteinases. The enzyme displays a strong activity towards both native and denatured proteins. It has no pronounced cleavage specificity. The predominant site of cleavage is the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked amino groups.

Application

- Isolation of high-molecular weight DNA
- Isolation of plasmid and genomic DNA
- Isolation of RNA
- Inactivation of RNase and DNase activities
- Study of structure of membranes² and human IgM³

Features

- Lyophilisate, activity: min. 8 DMC-U/mg* (min. 30 milliAnson-U/mg**)
- DNase and RNase not detectable
- Molecular weight (M_r): 28390¹ (AA sequence); 28500 (SDS-PAGE), Isoelectric point (pI): 8.9⁴
- pH range: 7.5 – 12.0⁴

Stability/Storage High thermal stability, particularly in the presence of Ca²⁺. Autolysis can occur at alkaline pHs, but this reaction is suppressed by Ca²⁺ ions. The enzyme is progressively and irreversibly denatured at acid pHs. Store lyophilisate at +2 °C to +8 °C. A solution is stable over a broad pH range (4.0 – 12.5, optimum pH 8.0) and over the temperature range of 25 to 65 °C. At pH 8 solutions will be stable for at least 12 months at 4 °C.

Activation 1 – 5 mM Ca²⁺ is required for activation. Activity is enhanced by incubation at elevated temperatures (i.e. 50 °C).

Inhibition Diisopropylfluorophosphate, phenylmethanesulfonyl fluoride² and mercury ions. Proteinase K is unaffected by metal-chelating agents and sulfhydryl inhibitors.

Reaction conditions Proteinase K 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.

*Unit definition: 1 DMC-unit catalyzes the cleavage of 1 mol peptide bond from dimethylcasein per minute at 25 °C, pH 7.0, expressed in terms of the appearance of new terminal amino groups.

**1 milliAnson U is defined as the amount of enzyme that liberates Folin-positive amino acids and peptides, corresponding to 1 µmol tyrosine under assay conditions in 1 minute using haemoglobin as substrate

¹Betzel, C., Pal, G.P. and Saenger, W. (1988) Eur. J. Biochem. 178, 155-171.

²Brdiczka, D. and Krebs, W. (1973) Biochim. Biophys. Acta 297, 203-212.

³Jehanli, A. and Hough, D. (1985) Molec. Immun. 22, 557-66.

⁴Ebeling, W., Hennrich, N., Klockow, M., Metz, H., Orth, H.D and Lang, H. (1974) Eur. J. Biochem. 7, 91-97.