PRODUCT INFORMATION

Proteinase K from Tritirachium album — Cat. No. 33752

Product description:

General

Proteinase K[1] 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 mMembranen[2] and human IgM[3]

Features

- Lyophilisate, activity: min. 8 DMC-U/mg* (min. 30 milliAnson U/mg**)
- DNase- and RNase activity not detectable
- Molecular weight (M_r): 28390[1] (AA-Sequence); 28500 (SDS-PAGE), Isoelectric point (pI): 8.9[4]
- pH range: 7.5 – 12.0[4]

Stability/ Storage

High thermal stability, particularly in the presence of Ca^{2+}. Autolysis can occur at alkaline pH values, but this reaction is suppressed by Ca^{2+} ions. The enzyme is progressively and irreversibly denatured at acid pH values. It is very stable in storage buffer (20 mM Tris-HCl, pH 7.4, 1 mM $CaCl_2$, 50% glycerol) at 4 °C or -20 °C. We recommend -20 °C as storage temperature.

Activation

1 - 5 mM Ca^{2+} is required for activation. Activity is enhanced by incubation at elevated temperatures (e.g. 50 °C).

Inhibition

Diisopropylfluorophosphate, phenylmethylsulfonylfluorid[2] and mercury ions. Proteinase K is unaffected by metal-chelating agents and sulfhydryl inhibitors.

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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

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

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

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

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

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