

Instruction Manual

Proteinase K from *Tritirachium album*, solution

Cat. No. 33755

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 pro-nounced 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

Features

- Ready to use in liquid form diluted in storage buffer (20 mM Tris-HCl, pH 7.4, 1 mM CaCl₂, 50 % glycerol).
- Can be stored at -20 °C still having the enzyme in liquid form
- Specific activity: >30 milliAnson-U/mg*, concentration: 20 mg/ml
- Free of Exonuclease, Endonuclease and RNase activity
- Molecular weight (M_r): 28390¹ (AA sequence); 28500 (SDS-PAGE),
Isoelectric point (pI): 8.9²
- pH range: 7.5 – 12.0²

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. It is very stable in the storage buffer at 4 °C or at -20 °C. We recommend as storage temperature -20 °C.

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

Inhibition Diisopropylfluorophosphate, phenylmethylsulfonyl 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.
Note: If necessary, to help the product to be fully soluble before use, heat the solution to 55 °C temperature and maintain the temperature for 15 – 45 min. After that cool and store at -20 °C. The product will retain its full activity.

***Unit definition:** 1 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.

²Ebeling, W., Hennrich, N., Klockow, M., Metz, H., Orth, H.D and Lang, H. (1974) Eur. J. Biochem. 7, 91-97.

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