

Proteinase K

Biochemical Research

Catalogue Number: MP023

Brief Description: Proteinase K is a serine endopeptidase with a broad spectrum of application. Evidence from crystal and molecular structure studies indicates the enzyme belongs to the subtilisin family with an active-site catalytic triad (Asp39-His69-Ser224). It is stable in a broad range of environments: pH, buffer salts, detergents (SDS), and temperature. In the presence of 0.1-0.5% SDS, proteinase K retains activity and will digest a variety of proteins and nucleases in DNA preparations without compromising the integrity of the isolated DNA.

Source: Tritirachium album limber

Molecular Weight: 28,900 daltons.

Formulation: 20 mg/ml solution containing 50% glycerol. Tested to be free of DNase and RNase.

Specificity: In addition to cleavage of peptide bonds, it is able to catalyze peptide amide hydrolysis. Proteinase K is inactivated by diisopropyl fluorophosphate (DFP) or phenyl methane sulphonyl fluoride (PMSF). Chelating agents such as citrate and EDTA have no effect on the enzyme activity.

Activity: ≥ 30 units/mg protein

Application: Proteinase K is very useful in the isolation of highly native, undamaged DNAs or RNAs, since most microbial or mammalian DNases and RNases are rapidly inactivated by the enzyme, particularly in the presence of 0.5 - 1% SDS.

- ✧ Useful for the proteolytic inactivation of nucleases during the isolation of DNA and RNA
- ✧ Removal endotoxins bound to cationic proteins such as lysozyme and ribonuclease A
- ✧ Useful for the isolation of hepatic, yeast, and mung bean mitochondria
- ✧ Determination of enzyme localization on membranes
- ✧ Treatment of paraffin embedded tissue sections to expose antigen binding sites for antibody labeling
- ✧ Digestion of proteins from brain tissue samples for prions in Transmissible Spongiform Encephalopathies (TSE) research

pH Optimum: 7.5 - 12, using denatured hemoglobin as substrate.

Pack Size: 1.0 mL

Storage/Stability: 2-8°C for up to 6 months. -20°C for long-term storage. Although calcium ions do not affect the enzyme activity, they do contribute to its stability when present at a concentration of 1 - 5 μ moles. An interesting characteristic of proteinase K is that it retains its activity in the presence of sodium dodecyl sulphate (SDS) or urea. (0.5-1% SDS and 1-4 M urea). Raising the temperature of the reaction from 37°C to 50°C-60°C can increase the activity several folds. A special feature of proteinase K is its ability to digest native proteins, thereby inactivating enzymes such as DNase and RNase without recourse to a denaturation process.

References:

Sweeney, P.J. and Walker, J.M., Burrell, M.M., Enzymes of molecular biology. Methods Mol. Biol. Towanam NJ , (1993) 16, 306.

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