Proteinase K

Product No.: 101-39450-01-6

Description

Proteinase K is a stable serine protease with broad substrate specificity. Proteinase K is a protease that cleaves at the carboxyl side of aliphatic, aromatic or hydrophobic residues and is commonly used to digest and inactivate DNase and RNase during in nucleic acid purification. 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, the name "Proteinase K". Evidence from crystal and molecular structure studies indicates the enzyme belongs to the subtilisin family with an active site catalytic triad (Asp39-His69-Ser224). 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.

Proteinase K is used for the destruction of proteins in cell lysates (tissue, cell culture cells) and for the release of nucleic acids, since it very effectively inactivates DNases and RNases.

Specification

Recommended usage: For DNA/RNA Experiment, for DNA/RNA Isolation
Physical State: Solid (lyophilized powder)
Appearance: White Powder
Molecular Mass: 28,930 Da (amino acid sequence); 28,500 Da (SDS-PAGE)
39.5 units/mg dry weight

Preparation Instructions

This product is soluble in water (1 mg/ml), yielding a clear colorless solution.
Recommended Concentration:
Stock Conc.: 10-20mg /mL
Working Conc.: 10-100 ug/mL

Applications

1. Mitochondria Isolation
2. Protein digestion for nucleic acid purification 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. The enzyme is typically used at 10-100 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 auto digest during long incubations.
3. Proteinase K has been used to remove endotoxins bound to cationic proteins such as lysozyme and ribonuclease A.
4. Determination of enzyme localization on membranes.
5. Treatment of paraffin embedded tissue sections to expose antigen binding sites for antibody labeling.
6. Remove nucleases for in situ hybridization.
7. Research on prions in Transmissible Spongiform Encephalopathies (TSE) and proposed diagnostic tests utilize Proteinase K digestion of proteins from brain tissue samples.
8. Protease footprinting by Proteinase K digestion can reveal protein-protein surface interactions.

Activators

1-5 mM Ca2+ is required for activation. When calcium is removed from the enzyme (addition of EDTA) 25% of the catalytic activity is lost. However, if the EDTA-Ca2+ 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 Ca2+ to the Ca2+-free enzyme.

Inhibitors

Proteinase K is inhibited by DIFP or PMSF (the latter used at final concentration 5 mM). 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-hloromercuribenzoate.

Notes

1. The activity of the enzyme is stimulated by 0.2- 1% SDS or by 1-4 M urea.
2. Ca2+ protects Proteinase K against autolysis, increases the thermal stability and has a regulatory function for the substrate binding site of Proteinase K.
3. Stable over a wide pH range: 4.0-12.5, optimum pH 7.5-8.0.

Storage and Stability

Store at -20 °C. It is stable for at least 2 years.