

Proteinasa K 20mg/ml, 1ml.



Description

Proteinase K is an endolytic protease that cleaves peptide bonds at the carboxylic sides of aliphatic, aromatic or hydrophobic amino acids.

The Proteinase K is classified as a serine protease. The smallest peptide to be hydrolyzed by this enzyme is a tetrapeptide.

Applications:

Isolation of genomic DNA from cultured cells and tissues

Removal of DNases and RNases when isolating DNA and RNA from tissues or cell lines

Determination of enzyme localization

Improving cloning efficiency of PCR products

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases confirmed by appropriate quality tests.

Concentration:

20 mg/ml

Source: Pichia pastoris cells with a cloned gene encoding Tritirachium album endolytic protease (Proteinase K).

Molecular Weight: 28.9 kDa monomer (6).



Definition of Activity Unit One unit of the enzyme liberates Folin-positive amino acids and peptides corresponding to 1 μ mol tyrosine in 1 min at 37°C using denatured hemoglobin as substrate. Enzyme activity is assayed in the following mixture: 0.08 M potassium phosphate (pH 7.5), 5 M urea, 4 mM NaCl, 3 mM CaCl2 and 16.7 mg/ml hemoglobin.

Storage Buffer:

The enzyme is supplied in: 50 mM Tris-HCl (pH 7.5), containing 5 mM calcium chloride and 50% (v/v) glycerol.

Inhibition and Inactivation:

Inhibitors: Proteinase K is not inactivated by metal chelators, by thiol-reactive reagents or by specific trypsin and chymotrypsiinhibitors. Phenylmethylsulfonyl fluoride and disopropyl phosphorofluoridate completely inhibit the enzyme.

Inactivated by heating at 95°C for 10 minutes.

Note Optimum activity at 50-55°C.

Rapid denaturation of enzyme occurs at temperatures above 65°C.

The recommended working concentration for Proteinase K is 0.05-1 mg/ml. The activity of the enzyme is stimulated by 0.2-1% SDS or by 1-4 M urea.

Ca2+ protects Proteinase K against autolysis, increases the thermal stability and has a regulatory function for the substrate binding site of Proteinase K.

Stable over a wide pH range: 4.0-12.5, optimum pH 7.5-8.0.