



Proteinase K

Cat No. N9011 1ml
Concentration: 20mg/ml
Store at -20°C

Features

- Ready-to-use solution
- Active in a wide range of reaction products

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.

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 chymotrypsin inhibitors. Phenylmethylsulfonyl fluoride and diisopropyl 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.



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