

Proteinase K

Molecular Biology Grade, For Research Use Only

Cat No. N9016

Size: 100mg

Specific Activity: ≥ 30 U/mg protein

Form: Contains proteinase K ($\geq 95\%$), lyophilized powder

Storage

-20°C recommended. The shelf life of proteinase K lyophilized powder is three years when stored sealed and dry below 4°C. Proteinase K lyophilized powder is shipped at room temperature.

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.

Features

- Recombinant proteinase K
- Active in a wide range of reaction products

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

DNase Activity: None detectable enzyme activity after 6 hrs incubation with λ DNA at 37°C.

RNase Activity: None detectable ribonuclease activity after 16 hrs incubation with RNA at 25°C.

Source

From yeast cells with cloned gene encoding genetically engineered *Engyodontium album* (*Tritirachium album*) endolytic protease.

Molecular Weight

29.3 kDa monomer.

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, pH 7.5 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.

Preparation Instructions

Stock solution can be prepared as 40-80mg/ml in dilution buffer [20 mM Tris-HCl (pH 7.4), 1 mM CaCl₂] or [20

mM Tris-HCl (pH 7.4), 1 mM CaCl₂, 2% Glycerol], sterilized using 0.22µm filter and supplied at final concentration of 20-40mg/ml in 50% Glycerol. Store in aliquots at wide temperature range from 24°C to -80°C. PES and PVDF membranes with low protein binding are recommended in sterile filtration.

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.