Proteinase K (Ultra Pure, Lyophilizated Powder)

CAS: 39450-01-6, E.C. 3.4.21.64

Synonyms: Peptidase K, Endoproteinase K, Endopeptidase K

Introduction

Proteinase K is a stable serine protease with broad substrate specificity. It degrades many proteins in the native state even in the presence of detergents. It is not activated by metal ions, chelating agents (for example, EDTA), sulfhydryl reagents, or by tripsin or chymotrypsin inhibitors. It is stable over a wide pH range (4–12.5), with optimal activity at pH 6.5–9.5. Activity can be stimulated by addition of denaturing agents (SDS and Urea). Rapid denaturation of the enzyme occurs at temperature above 70°C. Autolysis of the enzyme occurs increasingly at alkaline pH. However, Proteinase K is not completely inactivated by autolysis. Some enzyme fragments continue to maintain their complete proteolytic activity, even after extensive autolysis.

Proteinase K is frequently used in molecular biology applications to digest unwanted proteins, such as nucleases in DNA or RNA preparations from microorganisms, cultured cells, and plants. The enzyme is typically used at 50–200 ug/ml in nucleic acid preparations at pH 7.5–8.0 and 37~55°C. Incubation times vary from 30 minutes to 18 hours.

Reconstitute Proteinase K

Dissolve in 20 mM Tris HCl, pH 7.5, 10 mM calcium chloride, and 50% glycerol, then store at –20~8°C. When store at 2-8°C, Glycerol is optional. Storage at –20°C in the absence of glycerol can lead to precipitation of the Proteinase K. Bacterial growth can occur in solutions stored at 2–8°C over extended periods of time. Ca2+ can serve as a stabilizer to suppress autolysis.

Storage and Stability

Magen recommends storage at -20~8°C, When stored at 2~8°C, the product retains activity for at least 2 years.

Applications

DNA/RNA Isolation.

Ordering information

CAT.No.	Product Name	Package
C12100	Proteinase K, Ultra Pure, Lyophilizate, >30 units/mg of protein	1 g
C12101		10 g
C12102		100 g
PDB-1000	Buffer PDB (20mM Tris, pH 7.5, 10mM CaCl2, 50% glycerol, 0.1% Preservatives)	1000 ml

Specification

CAS No	39450-01-6
Molecular Weight	29.3kDa
Isoelectric Point	8.9
Appearance	White lyophilized powder
purity	95% (SDS-PAGE analysis)
Specific activity	⩾34Units/mg protein
Temperature characteristic	The effective activity temperature is 37-70°C, and the enzyme activity at 65°C is twice that at 25°C.
PH characteristics	4.0-12.0, the optimum range is ph7.5-11.5
Preservation Conditions	It is recommended to store at - 20°C to ensure the stability of activity to the greatest extent (normal temperature transportation or storage will not reduce enzyme activity) The shelf life is up to 3 years at -20°C and 2 years at 2~8°C.
Usage method	Prepare 20mg/ml with the solution, add proteinase K to the digestive solution or lysate until the final concentration is 50-200ug/ml, and incubate at 55~70°C. Proteinase K after reaction can be removed or inactivated by magnetic bead method, column method or phenol chloroform extraction. Protease K can be inactivated by incubation at 95°C for 3 minutes or 70°C for 15 minutes.
Nucleic acid residue detection	Qubit did not detect human DNA contamination not detected (real time PCR) bacterial DNA contamination not detected (16S universal primer PCR, 30cycles) fungal DNA contamination not detected (ITS Primer PCR)
Nuclease detection	DNase not detected RNase not detected Nickase not detected
Recommended application	Nuclear Nucleic acid extraction, Circulating DNA extraction, virus nucleic acid extraction,

Typical Experiments

- Isolation of gDNA: Dissolve the lyophilized Proteinase K at 20mg/ml in Buffe PDB and use it with HiPure Universal DNA Kit to isolation DNA from: 200ul mammalian blood, 2 x 10^6 cultured mammalian cells,10~20 mg mammalian tissue/mouse tail. 20ul of the reconstituted proteinase K solution is enough.
- Preparation of tissue sections for in situ hybridization: For some tissues, treatment of cytological sections with proteinase K will improve the likelihood that probes will reach cellular nucleic acids. The effectiveness of proteinase K treatment and the optimal concentration of proteinase K depend greatly on the kind of tissue and how it was fixed. For example, to treat blood vessel or myocardial tissue, Plenz et al (7) used the following concentrations of proteinase K: Cryosections: up to 2 g/ml; Paraffin-embedded sections: up to 20 g/ml; Methacrylate-embedded sections: up to 50 g/ml.