

Recombinant Proteinase K

Catalog No: #RE001

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Description

Product Name	Recombinant Proteinase K
Purification	DNase is not detected in quality control procedure of incubation 40 µg Proteinase K with 1 µg λ DNA at 37°C for 6 hours. RNase is not detected in quality control procedure of incubation 40 µg Proteinase K with 2 µg RNA at 37°C for 2 hours. Each lot was tested to ensure the absence of Nucleases, DNA and RNA.
Other Names	Peptidase K, Endoproteinase K, Endopeptidase K
Calculated MW	29.3 kDa
Formulation	The product is available as lyophilized powder. Please inquiry if buffered aqueous glycerol solution needed.
Storage	-20°Recommended

Application Details

Proteinase K is commonly used in molecular biology to remove protein contamination from preparations of highly native undamaged nucleic acid because it rapidly and effectively inactivates nucleases that might degrade the DNA or RNA even in the presence of denaturing reagents. Proteinase K is active in 1% Triton X-100 and fully active in 0.5% (w/v) SDS which denatures protein substrates to increase digestion rates. The enzyme works best at 2-20 mg/mL at pH 7.5-11.0, 37-70 °C and is usually denatured by subsequent phenol extractions. Incubation times vary from 30 minutes to 18 hours and proteinase K can auto digest during long incubations.

Product Description

Proteinase K is a broad-spectrum serine protease originally isolated from fungus *Engyodontium album*. The protease was named "Proteinase K" for its ability to digest Keratin. Crystal and molecular structure studies suggest that the

enzyme belongs to the subtilisin family characterized with a catalytic triad (Asp39-

His69-Ser224) in active site. Proteinase K has no pronounced cleavage specificity and preferential cleavage site is the peptide bond adjacent hydrophobic amino acids.

Specific activity: ≥34 units/mg of protein

Grade: Molecular Biology Grade

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

E.C.: 3.4.21.64

pI: 8.9

pH range: 4.5-12.0, optimum pH range 7.5-11.5

Temperature profile: Maximum activity at 70 °C; 37-70°C recommended.

Unit Definition: One unit is defined as the enzyme activity that produces 1 µmol of tyrosine per minute from casein at 37°C at pH 7.5. Refer to the certificate of analysis for specific values for the present lot.

Advantages: Our recombinant proteinase K is a mutant to the native protease that result in improved specific activity, higher yield and wider range of pH/temperature with optimal activity. The large scale recombinant preparation has advantages in lot-to-lot consistency, superior purity and cost-efficiency. DNA-free nature of recombinant Proteinase K made it well-suited in isolating DNA and RNA templates. Recombinant proteinase K is widely used for general digestion of proteins and Chemo-enzymatic peptide synthesis in molecular biology, molecular diagnostic and biopharmaceutical applications. The broad temperature profile of recombinant proteinase K helps with protein unfolding, easing the ability for proteinase K to breakdown those proteins.

Activators: 1-5 mM Ca²⁺: To stimulate proteinase K activity, 1-5 mM Ca²⁺ can be added. Optimization using

activators can increase proteinase activity significantly. Enzyme activity will be reduced by 25% when calcium is removed by addition of EDTA.

Enzyme activity will be reduced by 80% if the EDTA-Ca²⁺ complex is removed from the enzyme solution by gel filtration, while it can be partially

restored by addition of excess Ca²⁺.

Inhibitors: DIFP or PMSF: The enzyme is inactivated by DIFP or PMSF (PMSF used at final concentration 5 mM.). However, it is not inhibited by EDTA, iodoacetic acid, trypsin-specific inhibitor TLCK, chymotrypsin-specific inhibitor TPCK, and p-chloromercuribenzoate.

Preparation Instructions: Caution: The Molecular Biology Grade lyophilized powder is NOT sterile.

Stock solution can be prepared as 40-80 mg/ml in dilution buffer [20mM Tris-HCl

oO pH 7.4) 1 mM CaCl₂] or [20mM Tris-HCl oO pH 7.4 oO 1 mM CaCl₂ and 2% Glycerol], sterilized using a 0.22 µm filter and supplied at final concentration of 20-40mg/ml in 50% sterilized Glycerol. Store in aliquots at wide temperature range from 24°C to -80°C.

PES or PVDF membranes with low protein binding are recommended in sterile filtration. We offer irradiation sterilization option to bulk quantity order.

The Gamma irradiation procedure may cause slight enzyme activity loss. Please inquiry.

Stability: Lyophilized powder is stable for 3 years when stored dry below 4°C. Buffered aqueous glycerol solution is stable for 1 year when stored below 4°C.

Handling and Storage: Shipped at room temperature and storage recommended below 4°C.

Dilution buffer: 20mM Tris-HCl (pH 7.4), 1mM CaCl₂ or 20mM Tris-HCl (pH 7.4), 1mM CaCl₂ and 2% Glycerol.

Storage buffer: 20mM Tris-HCl (pH 7.4), 50% Glycerol.

Precautions and Disclaimer: This product is for R&D use only, not for drug, household, or other uses.

References

Note: This product is for in vitro research use only