LU5003

Proteinase K (Recombinant, Molecular Biology Grade)

Size	**1 g** (LU5003-1G) Bulk sizes available, please inquire
Form	Lyophilized powder
Activity	≥ 34 Anson units/mg of protein
Storage	Store lyophilized enzyme at -20°C. Store reconstituted enzyme at 4°C
Shelf life	36 months when stored at -20°C as lyophilized powder 12 months after reconstitution in appropriate buffer when stored at 4°C
E.C.	3.4.21.64
CAS No.	39450-01-6
Synonyms	Peptidase K, Endoproteinase K, Endopeptidase K

Properties

Source	Purified from yeast cells transformed with a cloned gene encoding genetically engineered *Engyodontium album (Tritirachium album)* endolytic protease.
Molecular mass	29.3 kDa
pI	8.9
Activity (pH range)	4.5-12.0 Optimum pH range 7.5-11.5
Activity (temperature range)	Maximum activity at 70 °C Recommended range 37-70°C
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.
Purity and Quality	DNase: not detected (incubation of 40 µg Proteinase K with 1 µg λ DNA at 37°C for 6 hours) RNase: not detected (incubation of 40 µg Proteinase K with 2 µg RNA at 37°C for 2 hours)

Product Description

Proteinase K is a broad-spectrum serine protease originally isolated from the fungus *Engyodontium album*. Proteinase K has no pronounced cleavage specificity, the preferential cleavage site is the peptide bond adjacent to hydrophobic amino acids. **Recombinant Proteinase K** is a mutant form of the native protease with improved activity, higher yield, and a wider activity range in terms of pH and temperature. Large-scale recombinant preparation guarantees high lot-to- lot consistency, superior purity, and cost-efficiency.

Applications

Proteinase K is commonly used in molecular biology to remove DNases and RNases from preparations of highly native undamaged nucleic acid and to non-specifically degrade proteins in lysates. 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 50-200 ug/mL at pH 7.5-11.0, 37-70 °C and is usually denatured by subsequent phenol extraction rounds. Incubation times vary from 30 minutes to 18 hours and Proteinase K can auto digest during long incubation times.

Preparation Notes

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

Recommended dilution buffer: 20mM Tris-HCl（pH 7.4), 1 mM $CaCl_2$ or 20mM Tris-HCl（pH 7.4), 1 mM $CaCl_2$ and 2% glycerol.

Prepare a stock solution by reconstituting the enzyme at 40-80 mg/ml in 40mM Tris-HCl and sterilize the enzyme solution using a 0.22 µm filter (e.g., Membrane Solutions Sterile Syringe Filters, Cat-No. SFPES030022S) and adding sterile glycerol to a final concentration of 50%. Store in aliquots at -20°C.

To stimulate proteinase K activity, 1-5 mM Ca^{2+} 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-Ca^{2+} complex is removed from the enzyme solution by gel filtration, while it can be partially restored by addition of excess Ca^{2+}. 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.

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

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