Datasheet for ABIN7211752

Proteinase K

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Overview

Quantity:	1 g
Target:	Proteinase K
Reactivity:	Tritirachium album
Host:	Yeast
Enzyme Type:	Recombinant
Biological Activity:	Active

Product Details

Purpose:	Proteinase K is a broad-spectrum serine protease originally isolated from fungus Engyodontium album.
Characteristics:	What is proteinase K used for? Proteinase K is commonly used for protein digestion, DNA extraction, RNA purification, direct PCR, and genotyping. Since it very effectively inactivates DNases and RNases, the recombinant proteinase K enzyme is added during nucleic acid preparations to isolate highly native, undamaged DNAs or RNAs; it is used in direct PCR kits for genotyping by destruction of proteins in cell lysates (tissue or cell culture cells) and for release of genomic DNA. 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. The proteinase K enzyme has no pronounced cleavage specificity and preferential cleavage site is the peptide bond adjacent hydrophobic amino acids. The proteinase K enzyme exhibits higher proteolytic activity in the presence of reducing agents

Product Details

	such as 5 mM DTT. Proteinase K is inhibited by serine protease inhibitors such as PMSF, DFP, and AEBSF. 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 7.5-8.0, 37 °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 incubation. The recombinant proteinase K enzyme is a mutant to the native protease, which gains higher specific activity and yield as well as wider pH and temperature range . The large scale recombinant preparation has advantage in lot-to-lot consistency, superior purity and cost-efficiency. DNA-free nature of recombinant Proteinase K made it well-suited in isolating PCR and RT-PCR templates.
Purity:	> 95 %
Endotoxin Level:	Less than 0.1 ng/µg (1 IEU/µg)
Biological Activity Comment:	Specific activity: ≥ 34 units/mg of protein.
Unit Definition:	One unit of the proteinase K enzyme is defined as the enzyme activity that produce 1 umol of tyrosine per minute from casein at 37°C at pH 7.5.

Target Details

Target:	Proteinase K
Abstract:	Proteinase K Products
Molecular Weight:	29.3 kDa
CAS-No:	39450-01-6

Application Details

Application Notes:	pI: 8.9. pH range: 4.5-12.0; high activity in the pH range of 7.5-11.5. Temperature profile: 37-70°C temperature range recommended; maximum activity at 70°C. Extinction coefficient: E1% = 14.2; 280 nm, 10 mM NaCl and 5 mM CaCl2, pH 8.0. Purity: >99.5% by reducing SDS-PAGE. Each lot of the proteinase K (powder and solution) was tested to ensure the absence of Nucleases and DNA. DNase is not detected in quality control procedure of incubation 40 µg Proteinase K with 1 ug λ DNA for 6 hours at 37°C. RNase is not detected in quality control procedure of incubation 40 µg Proteinase K with 2 ug RNA for 2

Application Details

	hours at 37°C. The preparation is considered as RNase and DNase free.
Comment:	Activators: 1-5 mM Ca2+. To stimulate proteinase K activity, 1-5 mM Ca2+ can be added. Optimization using activators can significantly increase proteinase activity. 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 proteinase K 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.
Reagent Preparation:	The molecular biology grade recombinant proteinase K, lyophilized powder is NOT sterile. It can be used to make molecular biology grade proteinase K solution, PCR grade proteinase K solution, NGS grade proteinase K solution, CHI grade proteinase K solution, and so on. The proteinase K stock solution can be prepared as a 20-40 mg/ml in 20 mM Tris-HCl buffer, sterilized using a 0.22 μm filter and supplied in 50% sterilized glycerol to 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.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Reconstitution:	The proteinase K enzyme is soluble in water (1 mg/mL), yielding a clear colorless solution.
Concentration:	500 U/μL
Buffer:	Storage buffer: 20 mM Tris-HCl, 1 mM CaCl2, 50% Glycerol, pH 7.4. Dilution buffer recommended: 20 mM Tris-HCl (pH 7.4), 1 mM CaCl2; or 20 mM Tris-HCl (pH 7.4), 1 mM CaCl2, and 2% Glycerol.
Storage:	-20 °C
Storage Comment:	2 years from date of receipt if stored at -20 to -70°C. 1 year from date of receipt if stored at room temperature. 1 year from date of receipt if stored at 2–8°C.
Expiry Date:	24 months