Proteinase K

blirt® DNA GDAŃSK

Proteinase K

A recombinant Proteinase K from *Tritirachium album* is a subtilisin-related serine protease. It is an endopeptidase with a very high specific activity and a broad spectrum of action. The enzyme is a 28.9 kDa protein expressed in *Pichia pastoris*. It's widely used for digestion of proteins, including DNases and RNases during nucleic acid preparations without compromising the integrity of the isolated DNA or RNA. It is active in a wide range of reaction conditions.

Features and advantages

- Supplied as highly purified lyophilized powder
- Specific activity: 30 U/mg protein
- Free of DNase and RNase activity
- Exhibits broad substrate specificity

Applications

- Inactivation of RNases and DNases during isolation of DNA or RNA from tissues, cell lines or cultured cells
- Removal of protein impurities
- Improving cloning efficiency of PCR products
- Determination of enzyme localization on membranes
- Removal of nucleases for *in situ* hybridization

Usage

The enzyme is typically used at 50–200 µg/ml in nucleic acid preparations at pH 7.5–8.5 and 37–55°C. Incubation times vary from 30 minutes to 18 hours.

Additional considerations

- The Proteinase K cleaves proteins preferably behind hydrophobic amino acids. The smallest peptide to be hydrolyzed is a tetrapeptide.
- Working pH range is 4.0–12.0 with optimum activity at pH 7.5–8.5. Full activity is maintained over several hours over a pH range of 6.5–10.0.
- Working temperature range is 20–65°C with optimum activity at 50–56°C.
- The enzyme is stimulated by addition of denaturing agents 0.2–1% SDS or 1–4M urea. It exhibits prolonged stability due to the presence of Ca^{2+} (1–6 mM), which protects enzyme from autolysis and increases its thermal stability.
- The enzyme is not inactivated by chelating agents (e.g. EDTA), chaotropic salts, detergents (e.g. 1% SDS, 14 M urea), metal ions, thiol reagents or trypsin-specific inhibitors.
- Proteinase K is usually denatured by subsequent phenol extractions. It can be also inactivated by heating above 65°C or using inhibitors such as PMSF or DIFP.

Storage buffer

- 50 mM Tris-HCl (pH 8.0), 5 mM $CaCl_2$ for storage at 2–8°C
- 50 mM Tris-HCl (pH 8.0), 5 mM $CaCl_2$, 50% glycerol (v/v) for storage at -20°C.

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Component	RP101B	RP102B
Proteinase K 30 U/mg	250 mg	1000 mg

Additional information

Quality control
The absence of endo-, exodeoxyribonucleases and ribonucleases has been confirmed in appropriate quality tests.

Unit definition
One unit is defined as an amount of enzyme that liberates Folin-positive amino acids and peptides corresponding to 1 µmol tyrosine in 1 min at 37°C using denatured hemoglobin as substrate. Enzyme activity is assayed in the following mixture: 80 mM potassium phosphate (pH 7.5), 5 M urea, 4 mM NaCl, 3 mM $CaCl_2$ and 16.7 mg/mL hemoglobin.

Storage conditions
Lyophilized Proteinase K or solutions in 50 mM Tris-HCl (pH 8.0), 5 mM $CaCl_2$, 50% glycerol (v/v) can be stored at -20°C for at least 12 months from the date of purchase. Enzyme solutions in 50 mM Tris-HCl (pH 8.0), 5 mM $CaCl_2$ are stable for 4–8 weeks at 2–8°C.

Shipping conditions
Shipping on dry or blue ice.

For research use only

Date of purchase

Warranty
12 months from the date of purchase

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