Product Data Sheet

Product Name: Proteinase K
Cat. No.: GP10161

Chemical Properties

Cas. No.	39450-01-6		
Formula		M.Wt	29.3 kDa
Solubility	H_2O : 25 mg/mL (Need ultrasonic)	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	Proteinase K		

Protocol

This protocol is used in the extraction of genomic DNA from mouse tail with Proteinase K.
1. Place an approximately 0.5cm mouse tail into a 1.5 ml microcentrifuge tube.
2. Add 500 µl of lysis buffer (For example: 50 mM KCl, 50 mM Tris-HCl (pH 8.0), 2.5 mM EDTA,0.45% NP-40, 0.45% Tween-20).
3. Add 2.5 µl Proteinase K (20 mg/ml) to the tubeand mixthe solution.
4. Incubate overnight at 55°C.
   Optional step:Incubate for an additional 1 hour at 65°C.
5. Vortex the tube and spin down for 10 seconds at 13,000 rpm to collect cell debris.
6. Use 1 µl from the top part of the supernatant per 50 µl of PCR mix(for genotyping).

Background

Proteinase K is a broad-spectrum serine protease and our product is extracted from Pichia pastoris cells with cloned gene encoding Engyodontium album (Tritirachium album) endolytic protease. It is a highly reactive protease frequently used for digesting various proteins and enzymes (including endonuclease, exonuclease, DNase or RNase) .Therefore, it is usually used in DNA preparations without impairing the integrity of the isolated DNA. It has a superior performance under a broad range of conditions: pH, buffer, detergents (such as SDS), chelator (such as EDTA), and temperature. Proteinase

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K hydrolyzes peptide bonds preferentially adjacent to carboxyl group of hydrophobic amino acids (aliphatic, aromatic, and others).

Except the isolation of genome, it can also take a job in detection of enzyme localization or removal of enzymes from DNA to improve cloning efficiency.

Appropriate working concentration of proteinase K is always among the range of 0.05 to 1 mg/mL. The activity of the enzyme can be stimulated by 0.2 to 1% SDS or by 1 to 4 mol urea. It is activated by calcium (1-5mM), although calcium ions do not affect the enzyme activity, but it contributes to the thermal stability and protects the proteinase from autolysis. Proteinase K has two binding sites for Ca^{2+}, which are located close to the active center, but are not directly involved in the catalytic mechanism. So calcium ion has a regulatory function for the substrate binding site of proteinase K. The enzyme is inactivated by DIFP or PMSF. However, it is not inhibited by EDTA, iodoacetic acid, trypsin-specific inhibitor TLCK, chymotrypsin-specific inhibitor TPCK, and p-chloromercuribenzoate.

We recommend an optimum pH of 7.5 to 8.0 and optimum temperature at 50 to 55°C. Rapid denaturation will occur at temperatures above 65°C. You can hold it under 95°C for 10 min as a heat inactivation.

References:
[1]. Kraus, E; et.al. Proteinase K from the Mold Tritirachium album limber, Specificity and Mode of Action. Z. Physiol. Chem., 357:939; 1976.
[2]. Jany,KD, et al. Amino Acid Sequence of Proteinase K from the Mold, Tritirachium albumlimber. Proteinase K; a Subtilisin-related Enzyme with Disulfide Bonds. FEBS Letter, 199,139.1986.

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