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PRODUCT INFORMATION
Proteinase K (Lyophilized Form)

Product: Proteinase K (Lyophilized Form)
Grade: Molecular Biology Grade
Code: PC0712-100g/1g
Molecular Weight: 28.8 kDaltons
Concentration: Not applicable

Description

Proteinase K is a recombinant enzyme that expressed in *Pichia pastoris* which is originally isolated from the mold *Tritirachium album.* It is highly active, subtilisin-related serine endopeptidases that does not exhibit any pronounced cleavage specificity. Thus, Proteinase K is treated as a universal tool for nucleic acid template preparation.

The recombinant form and native protease are having identical amino acid sequence and molecular structure. Compared to native protease, the recombinant enzyme guarantees an enzyme of outstanding reliability and purity meeting all requirements of molecular and diagnostic tests. The recombinant form of Proteinase K enzyme maximizes the yield of target nucleic acids. The lyophilized form experience stability and flexibility at ambient temperature during shipment.

Application:

- Digest native proteins very efficiently.
- Inactivate endogenous RNases and DNases rapidly during nucleic acid isolation.
- Isolation of native RNA and DNA from tissues and cell lines.
- Promotes cell lysis by activating a bacterial autolytic factor.
- Analysis of membrane structures by modifying proteins and glycoproteins on cell surfaces.
- Removal of cellular debris during the preparation of colony lifts.
- Treatment of tissue sections to ensure efficient probe infiltration during *in situ* hydridization.

Storage Temperature

Store at -15 to -25°C within specification range for 24 months. Avoid exposure to frequent temperature changes. See the expiration date on the stickers of product item.
Store at 2 to 8°C within specification range for 12 months.
Lyophilized form enzyme store at room temperature for at least 6 months.
Lyophilizate dissolved in water stable within 60 days when stored at 4°C.

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Unit Definition

One unit of Proteinase K hydrolyzes urea-denaturated hemoglobin producing color equivalent of 1µmol tyrosine per 1 minute at 37°C and pH = 7.5 (Folin $ Ciocalteu's method), 1U = 1mAnsonU.

Volume activity (37°C, hemoglobin): ≥ 30 U/mg lyophilizate
Specific activity (37°C, hemoglobin): ≥ 40 U/mg protein

Protein content:	≥70% (determined by measuring absorbance at 280nm.)
DNA content:	≤10pg/mg by qPCR
Exonucleases:	1µg of Hind III-digested λ DNA is incubated with 50µg Proteinase K for 16 hours at 37°C. No DNA degradation is detectable.
Endonucleases:	1µg of pUC19 DNA is incubated with 40µg Proteinase K for 16 hours at 37°C. No DNA degradation is detectable.
Ribonucleases:	2µg of rRNA from *E.coli* is incubated with 20µg of Proteinase K for 4 hours at 37°C. No RNA degradation is detectable.

Reconstitute Buffer

50mM Tris-HCl, pH 7.5-8.0; 3mM $CaCl_2$ for immediate use.
50mM Tris-HCl, pH 7.5-8.9; 3mM $CaCl_2$; 50% glycerol for long-term storage at -20°C.
Suggested concentration: 20 mg/ml

Suggested Buffer

The buffer for Proteinase K is vary depends on different applications. Proteinase K is always stable and very active in buffers that contain denaturing reagents such as urea, sodium dodecyl sulfate (SDS), and guanidinium salts.

Suggested pH and Temperature

Proteinase K is stable from pH 4.0 to pH 12.5. The enzyme retains full activity for several hours when incubated at pH 6.5–9.5. The optimum temperature for Proteinase K activity is 65°C. However, it is rapidly denatured at temperatures above 65°C.

Activators

Denaturing agents such as SDS and urea can stimulate Proteinase K activity.

Inhibitors

Proteinase K can be inhibited by diisopropyl fluorophosphates and phenulmethylsulfonyl fluoride (PMSF). It can be inactivated by mercury ions. Pefabloc SC* and Pefabloc PLUS* are specific, irreversible, non-toxic inhibitors of Proteinase K.

Autolysis

Autolysis of the Proteinase K enzyme occurs more rapidly at alkaline pH. However, proteinase K is not completely inactivated by autolysis. Some enzyme fragments retain complete proteolytic activity.

Suggested Procedure

Isolation of Nucleic Acid

1. Use 40µl of the 20 mg/ml of Proteinase K to each sample. If paired with lysis buffer of commercial DNA/RNA extraction kit, use 20µl of 20 mg/ml of Proteinase K to each sample.
    a. 200µl mammalian blood
    b. 200µl buffy coat
    c. 25-200mg mammalian tissue
    d. 25-50mg formalin-fixed paraffin-embedded tissue section
    e. 10^9 bacteria cells
    f. 10^5 cultured cells

Isolation of Genomic DNA from Mammalian Tissue

1. Use 1ml of the digestion buffer that contain 100 µg/ml of Proteinase K and 0.5% SDS buffer to each sample. Incubate the mixture for 12-18hours at 50°C. The samples can be:
    a. 80-100mg mammalian tissue which is minced or frozen
    b. 10^8 cultured mammalian cells

Isolation of Cytoplasmic RNA from Cultured Cells

1. Lyse cells in a buffer containing 0.5% Nonidet P-40.
2. Centrifuge the lysate and transfer the supernatant to a clean tube.
3. Add 4µl of 20% SDS and mix the tube immediately.
4. Add 2.5µl of 20 mg/ml Proteinase K and incubate for 15 minutes at 37°C.

Preparation of tissue sections for *in situ* hybridization

1. Improvement with Proteinase K treatment:
    a. Cryosections: up to 2 µg/ml
    b. Paraffin-embedded sections: up to 20 µg/ml
    c. Methacrylate-embedded sections: up to 50 µg/ml