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PRODUCT INFORMATION
Proteinase K (Solution Form)

Product: Proteinase K (Solution Form)

Grade: PCR Grade

Code: PC0712-1ml

Molecular Weight: 28.8 kDaltons

Concentration: 20 mg/ml

Lot No.:

Expiry Date:

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 as well as the solution form experience stability at room temperature and flexibility during shipment at room temperature.

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.

Unit Definition

One unit is the enzyme activity which releases folin positive amino acids and peptides equivalent to 1µmol of tyrosine in 1 minute under the test conditions.
One unit is the enzyme activity which cleaves 8mmol Chromozym TRY in 1 minute at 25°C.

Volume activity (37°C, hemoglobin): ≥ 24 U/mg lyophilizate
Specific activity (37°C, hemoglobin): ≥ 30 U/mg protein
Volume activity (25°C, Chromozym): ≥ 2 U/mg lyophilizate
Specific activity (25°C, Chromozym): ≥ 2.5 U/mg protein

Unspecific endonucleases (MWM III DNA):	Not detectable in up to 200µg after 16 hours incubation at 37°C.
Nicking activity (pBR322 DNA):	Not detectable in up to 200µg after 16 hours incubation at 37°C.
Ribonucleases (MS2 RNA):	Not detectable in up to 40µg after 16 hours incubation at 37°C.
DNA (Threshold®):	≤10 pg.mg enzyme
Bioburden:	≤125 CFU/g

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 18 months.
Store at room temperature for at least 12 months.

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.

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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