Thermo SCIENTIFIC

PRODUCT INFORMATION

Proteinase K (recombinant), PCR grade

#EO0492 5 x 1 mL

Lot: _ **Expiry Date: _**

Concentration: >600 U/mL (~20 mg/mL)

Store at -20°C

In total 5 vials.

Description

Proteinase K is an endolytic protease that cleaves peptide bonds at the carboxylic sides of aliphatic, aromatic or hydrophobic amino acids.
The Proteinase K is classified as a serine protease (1).
The smallest peptide to be hydrolyzed by this enzyme is a tetrapeptide.

Applications

- Isolation of genomic DNA from mouse tail.
- Isolation of genomic DNA from cultured cells.
- Removal of DNases and RNases when isolating DNA and RNA from tissues or cell lines (2, 3).
- Determination of enzyme localization (4).
- Improving cloning efficiency of PCR products (5).

Source

Pichia pastoris cells with a cloned gene from *Tritirachium album.*

Molecular Weight

28.9 kDa monomer (6).

Definition of Activity Unit

One unit of the enzyme 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: 0.08 M potassium phosphate (pH 7.5), 5 M urea, 4 mM NaCl, 3 mM $CaCl_2$ and 16.7 mg/mL hemoglobin.

Storage Buffer

The enzyme is supplied in: 10 mM Tris-HCl (pH 7.5), containing calcium acetate and 50% (v/v) glycerol.

Inhibition

- Phenylmethylsulfonyl fluoride and diisopropyl phosphorofluoridate completely inhibit the enzyme (1).
- Proteinase K is not inactivated by metal chelators, by thiol-reactive reagents or by specific trypsin and chymotrypsin inhibitors.

Note

- The recommended working concentration for Proteinase K is 0.05-1 mg/mL. The activity of the enzyme is stimulated by 0.2-1% SDS or by 1-4 M urea (3).
- Ca^{2+} protects Proteinase K against autolysis, increases the thermal stability and has a regulatory function for the substrate binding site of Proteinase K (7).
- Stable over a wide pH range: 4.0-12.5, optimum pH 7.5-8.0 (8).

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 40 µg of Proteinase K with 1 µg of pUC19 DNA for 4 hours at 37°C.

Ribonuclease Assay

No detectable RNA degradation after incubation of 80 ng of 2 kb RNA transcript with 40 µg of Proteinase K for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No degradation of single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 40 µg of Proteinase K for 4 hours at 37°C.

Quality authorized by:  Jurgita Zilinskiene

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References

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3. Hilz, H., et al., Stimulation of proteinase K action by denaturing agents: application to the isolation of nucleic acids and the degradation of "masked" proteins, Eur. J. Biochem., 56, 103-108, 1975.
4. Brdiczka, D. and Krebs, W., Localization of enzymes by means of proteases, Biochim. Biophys. Acta, 297, 203-212, 1973.
5. Crowe, J.S., et al., Improved cloning efficiency of polymerase chain reaction (PCR) products after proteinase K digestion, Nucleic Acids Res., 19,184, 1991.
6. Jany, K.D., et al., Amino acid sequence of Proteinase K from mold *Tritirachium album* Limber Proteinase K – a subtilisin related enzyme with disulfide bonds, FEBS Lett., 199, 139-144, 1986.
7. Bajorath, J., et al., The enzymatic activity of proteinase K is controlled by calcium, Eur. J. Biochem., 176, 441-447, 1988.
8. Ardelt, W., Laskowski, M.Jr., Turkey ovomucoid third domain inhibits eight different serine proteinases of varied specificity of the same ...Leu18-Glu19... reactive site, Biochemistry, 24, 5313-5320, 1985.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

SAFETY INFORMATION

Proteinase K

Xn Harmful

Hazard-determining components of labeling:
Proteinase, Tritirachium album serine

Risk phrases

R42 May cause sensitization by inhalation.

Safety phrases

S23 Do not breathe gas/fumes/vapor/spray.
S36 Wear suitable protective clothing.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S60 This material and its container must be disposed of as hazardous waste.