Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Proteinase K

#EO0492 5 x 1 ml

Lot: **Expiry Date:**

Concentration: 900 u/ml (18.9 mg/ml)

Store at -20°C

ISO 9001 ISO 14001
www.fermentas.com

Description

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

Source

Pichia pastoris cells with a cloned gene encoding *Tritirachium album* endolytic protease (Proteinase K).

Molecular Weight

28.9 kDa monomer (2).

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 (3,4).
- Determination of enzyme localization (5).
- Improving cloning efficiency of PCR products (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

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

Inhibition and Inactivation

- Inhibitors: Proteinase K is not inactivated by metal chelators, by thiol-reactive reagents or by specific trypsin and chymotrypsin inhibitors. Phenylmethylsulfonyl fluoride and diisopropyl phosphorofluoridate completely inhibit the enzyme (1).
- Inactivated by heating at 95°C for 10 min.

Note

- Optimum activity at 50-55°C.
- Rapid denaturation of enzyme occurs at temperatures above 65°C.
- 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 (4).
- Ca^{2+} protects Proteinase K against autolysis, increases the thermal stability and has a regulatory function for the substrate binding site of Proteinase K (8).
- Stable over a wide pH range: 4.0-12.5, optimum pH 7.5-8.0 (7).

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 0.5 units of Proteinase K with 1 µg of pBR322 DNA in 50 µl of buffer (10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$) for 4 hours at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 0.5 units of Proteinase K with 1 µg of sonicated *E.coli* [^{3}H]-DNA in 50 µl of buffer (10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$) for 4 hours at 37°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 0.5 units of Proteinase K with 1 µg of [^{3}H]-RNA in 50 µl of buffer (10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$) for 4 hours at 37°C.

Quality authorized by: Jurgita Zilinskiene

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References

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3. Wiegers, U. and Hilz, H., A new method using 'proteinase K' to prevent mRNA degradation during isolation from HeLa cells, Biochem. and Biophys. Res. Commun., 44, 513-519, 1971.
4. 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.
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Related Product

- RNase A/T1 Mix #EN0551
- Water, nuclease-free #R0581

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.fermentas.com for Material Safety Data Sheet of the product.

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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.