MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

Catalog Number: 193504, 193981
Proteinase K

Molecular Weight: 28,930 daltons
CAS # : 39450-01-6
Synonym: 3.4.21.14
Physical Description: White to off white lyophilized powder
Description: A highly active stable endopeptidase with a broad spectrum of action was isolated by E. Merk's Darmstadt Biochemical Research Department in 1970 from a culture filtrate of the fungus, Tritirachium album Limber. This fungus is able to grow on keratin (e.g., wool, horn particles) as the sole source of carbon and nitrogen. The isolated protease was, therefore, given the K designation.
For the isolation of native, high molecular weight nucleic acids: DNA, RNA
Following isolation and chromatographic purificaiton Proteinase K becomes a homogenouse crystallizable protein.
Isoelectric point (isoelectric focusing): pI 8.9
pH optimum (denatured hemoglobin as substrate): pH 7.5-12.00
Solubility: Soluble in water
Applications

– ***Isolation of native high molecule weight nucleic acids (DNA, RNA)***

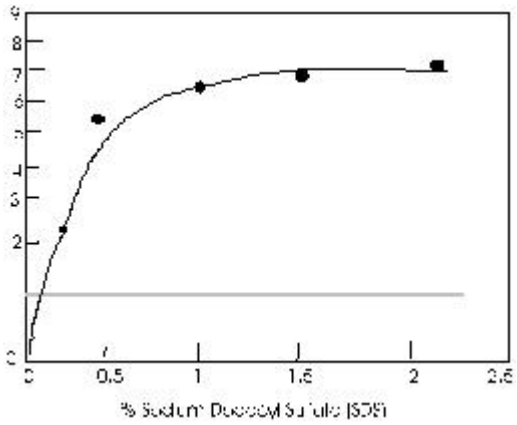
Proteinase K preparations are free of ribonuclease (RNase) and deoxyribonuclease (DNase). After 60 minutes incubation of radio labeled adenovirus-DNA with Proteinase K at 37ºC, only 0.005% of the original radioactivity was found in the acid -soluble portion. DNases and RNases from most microorganisms and mammalian cells are rapidly inactivated by Proteinase K, particularly in the presence of 0.5-1.0% of SDS. Addition of Proteinase K during the cell digestion enables the isolation of native undamaged high molecular DNA or RNA. This method has been established as a standard method, as documented in numerous publications and text books.

– ***Analysis of membrane structure***

Proteinase K is very useful in the specific modificaiton of proteins and glycoproteins on cell membranes.

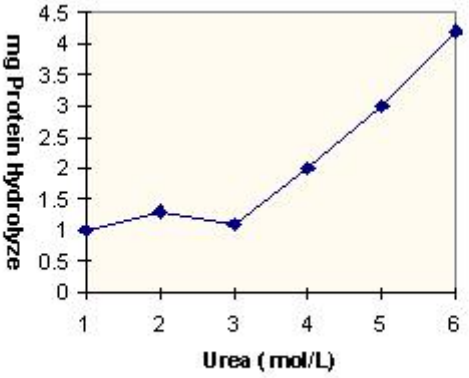
– ***Structural investigaitons on proteins***

Because of the cleavage specificity of Proteinase K , characteristic fragments of proteins are obtained which are useful in determing the structure and function of proteins, particullarly enzymes.
Specificity: Proteinase K cleaves peptide bonds mostly after the carboxyl group of N-substituted hydrophobic aliphatic and aromatic amino acids, as shown by specificity trials with amino acid-4-nitroacilides. Thus, it shows similarities with alkaline Asperigillus proteases. However, unlike the latter, Protease K also cleaves peptide amides, comparable to the alkaline serine-proteases from Bacillus species. The specificity of ester cleavage is also high.
Inhibition: Proteinase K belongs to the group of serine proteases with an easily esterifed serine fragment at the active center and, as with other proteases in this group, e.g. trypsin, chymotrypsin, is inactivated by diisopropylfluorophosphate or phenylmethane sulfonyl fluoride. Also inhibited by AEBSF and trypsin inhibitor.
Metallic ion complexing agents, e.g., chelate formers such as EDTA and sulfhydryl reagents, have no effect on the activity of Proteinase K.
Activity
Toward denatured hemoglobin as substrate, this highly purified Proteinase K demonstrates a specific activity of 32mAnson units/ mg and is, therefore, 6 times as active, weight for weight, as the Streptomyces protease "pronase" and about 3 times as active as beef trypsin.



A remarkable property of Proteinase K is its ability to rapidly deactivate native proteins , particularly enzymes by hydrolysis. Addition of either 0.5-1% of sodium dodecyl sulfate or 1-4 mmol/l of urea increases the activity, because the substrates are more easily attacked when denatured. Proteinase K itself denatured much more slowly by these agents.

Proteinase K Activity

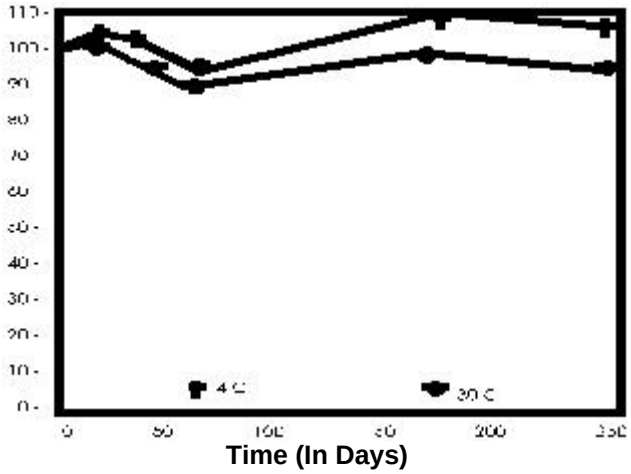


Activation of proteolytic activity of Proteinase K with sodium dodecyl sulfate (SDS) and urea. Substrate : beef serum albumin .

Stability

The enzyme is stable for a number of years in the solid form when stored dry in an airtight container at approximately 4ºC. Aqueous solutions containing Ca2 + (1-5 mmol/l) are stable for several weeks at pH 4.0-11.5 at room temperature and are resistant to autolysis. Compared with denaturation agents such as dodecyl sodium salt (SDS) or urea, Proteinase K is extrodinarily stable.

More recently Proteinase K has become available in solution form stable for many months even at room temperature (see adjacent chart)/ This convenient, ready-to-use form omits the steps needed to bring a specified quanity into solution and avoids the potential hazard of inadvertent contact. 600 mAnson units of activity per 10ml allows the user to pipet precise aliquots required for protein digestion.



Assay

Method: Proteinase K hydrolyzes hemoglobin denatured with urea, and liberates Folin Postive amino acids and peptides, which are determined as tyrosine equivalents. 1 unit releases 1 umole Folin positive amino acid in 10 minutes at 37ºC, pH 7.5, using denatured hemoglobin as substrate.

Reagents:

– 0.05 N HCl - Dilute 0.82 ml concentrated HCl to 200 ml with reagent grade water.
– 0.5 M NaOH - Dissolve 4.0 gm NaOH in 200 ml reagent grade water.
– Buffer-Substrate - Dissolve 2.0 gm hemoglobin in 35 ml reagent grade water, add 36.0 gm urea and 16 ml 0.5 NaOH. Stir for 30-60 minutes at room temperature. Add 0.618 gm boric acid and stir. Adjust the pH to 7.5 with 5 N HCl and q.s. to 100 ml
– Tyrosine standard (2.5 nmol/L) - Dissolve 45.3 mg tyrosine in 100 ml of 0.05 N HCl.

– 0.3 M Trichloroacetic acid - Dissolve 9.8 gm trichloroacetic acid in 200 ml reagent grade water.
– Folin Reagent - Add 10 ml Folin-Ciocalteus Phenol Reagent to 20 ml reagent grade water.

Enzyme:
Dissolve 10 mg lyophilized material in 1 ml reagent grade water. Prepare a 1:1000 dilution with water immediately before use.
Procedure:
Label clear glass test tubes for blank, standard, and test. Add 2.5 ml buffer-substrate and incubate for 5 minutes at 37ºC. Start reaction by adding 0.2 ml tyrosine standard to the standard tube, 0.2 ml of sample to the test, and 0.2 ml of 0.05 N HCl to the blank. Incubate for 10 minutes at 37ºC. Stop reaction by the addition of 5.0 ml trichloroacetic acid. Mix, then add 0.2 ml of sample to the blank and standard, and add 0.2 ml of 0.05 N HCl to the test. Mix and let stand for 10 minutes at room temperature, filter and pipette into test tubes 1.0 ml of filtrate, 2.0 ml of 0.5 N NaOH, and 0.6 ml of Folin Reagent. Mix well. Let stand for 15 minutes and read A_{578} nm.
Calculation:
Units/mg = ((0.5 umoles tyrosine) / (0.2 ml X 10 min)) X ((A_{578} of sample - A_{578} of blank) / (A_{578} of standard)) X Dilution
Availability:

Catalog Number	Description	Size
193504	Proteinase K	25 mg 100 mg 250 mg 500 mg 1 g
193981	Proteinase K, molecular biology reagent	5 mg 10 mg 25 mg 100 mg 500 mg 1 g

Inactivation of DNases and RNases for the isolation of nucleic acids[1-4]; structure-function investigations of proteins.[5,6]
References :

– Orth,H.D. : *Konakte* (*Merck*), **v. 3/76**, 35 (1976).
– Ebeling, W., et al., *Eur. J. Biochem.*, **v. 47**, 91 (1974)
– Capesios, J., et al., *FEBS-Letters*, **v. 110**, 184 (1980)
– Rauber, N.R.K., et al., *Z. Naturforsh.*, **v. 33c**, 660 (1978)
– Williamson, J., et al, *Biochem. J.*, **v. 167**, 731 (1977)
– Morris, G.E., et al ., *Biochem. J.*, **v. 228**, 375 (1985)
– Bajorath, J., et al., *Eur. J. Biochem.*, **v. 176**, 441-447 (1988).
– Betzel, C., *Eur. J. Biochem.*, **v. 178**, 155-171 (1988).
– Gross-Bellard, M., et al., *Eur. J. Biochem.*, **v. 36**, 32-38 (1973).
– Hilz, H., et al., *Eur. J. Biochem.*, **v. 56**, 103 (1975).
– Jany, K.D., Lederer, G. and Mayer, B., *FEBS Lett.*, **v. 199**, 139-144 (1986).
– Jany, K.D. and Mayer, B., *Biol. Chem. Hoppe-Seyler*, **v. 366**, 485 (1985).
– Kraus, E. and Femfert, U., *Z. Physiol. Chem.*, **v. 357**, 937 (1976).
– Lizardi, P.M., et al., *Anal. Biochem.*, **v. 98**, 116 (1979).