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Proteinase K

Catalog Number: PK01-1, PK01-2

Table 1. Kit Components and Storage

Kit Component	PK01-1	PK01-2	Storage	Stability
Proteinase K	100 mg	500 mg	4 °C	The product is stable for 12 months when stored as directed.

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)
- Analysis of membrane structure
- Structural investigaitons on proteins

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.

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 A578 nm.

Calculation:

Units/mg = ((0.5 umoles tyrosine) / (0.2 ml X 10 min)) X ((A578 of sample - A578 of blank) / (A578 of standard)) X Dilution