Catalog Number: 151972

Protease, Strain V8

Molecular Weight: 27,000[7] or 27,700 by sedimentation equilibrium.

CAS # 9001-92-7

Synonym: Endoproteinase Glu-C

E.C. 3.4.21.19

Source: *Staphylococcus aureus* strain V8

Preparation: The enzyme was isolated, by the method of Drapeau et al.[4], from the culture filtrate of S. aureus V8 strain. The preparation is homogenous on ultracentrifugation and disc electrophoresis.

Extinction Coefficient: $E^{1\%}_{280} = 4.26$[10]

Optimum pH: 4.0 and 7.8 with hemoglobin substrate.[4]

Inhibitors: Diisopropyl fluorophosphate (DFP) and monovalent anions such as F^-, Cl^-, Br^-, CH_3COO^- and NO_3^-.[10]

Physical Description: Off-white lyophilized solid

Activity: Approximately 500 units/mg

Unit Definition: One unit will hydrolyze casein to change A_{280} 0.001 per minute, pH 7.8, at 37°C.

Solubility: Soluble in aqueous buffers (such as 10 mM Tris, pH 7.8 at 1 mg/ml). Solutions can be stored refrigerated for up to 1 month or aliquoted and stored at -20°C for approximately 6 months.

Description: Protease V8 is used for selective cleavage of proteins for amino acid sequence determination[2] or peptide mapping.[3,9] The *Staphylococcus* strain V8 protease specifically cleaves peptide bonds on the carboxyl (COOH) terminal side of aspartic and glutamic acid residues.[4]

Assay:

Method: Enzyme activity is determined by the casein digestion assay described by Drapeau, et al.[4]

Reagents:

1. 1% Casein in 0.05 M Tris·PO_4 buffer, pH 7.8: Dissolve 1 gram Hammersten grade casein in 50 ml 0.01 N NaOH with gentle heating and stirring. Add 40 ml reagent grade water and 5.0 ml 1.0 M Tris. Adjust pH to 7.8 with H_3PO_4 and q.s. to 100 ml.
2. 10% Trichloroacetic acid (TCA)

Enzyme:

Dissolve at 1 mg/ml in reagent grade water.

Procedure:

Equilibrate a series of tubes with 5.0 ml of 1% casein at 37°C for 5 minutes. At zero time add 10 ul or 20 ul of enzyme. Mix. Include a reagent blank. Exactly ten minutes after adding sample, stop reaction by adding 5.0 ml TCA. Mix. Allow tubes to stand ten minutes and then filter. Read A_{280} of the filtrate.

Calculation:

$$\text{Units/mg} = \frac{A_{280}\,(\text{test}) - A_{280}\,(\text{reagent blank})}{10\ \text{minutes} \times \text{mg of enzyme in reaction}}$$

Click Here for a list of protease inhibitors offered by MP Biomedicals and general protease inhibitor information.

References:

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3. Cleveland, D.W., et al., "Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis." *J. Biol. Chem.*, **v. 252**, 1102 (1977).
4. Drapeau, G.A., Boily, Y. and Houmard, J., "Purification and properties of an extracellular protease of *Staphylococcus aureus*." *J. Biol. Chem.*, **v. 247**, 6720 (1972).
5. Kunitz, M., J. Gen. Physiol., v. 30, 291 (1947).
6. Drapeau, G., "Protease from *Staphylococcus aureus*." in *Methods in Enzymology*, XLVB, (Lorand, L., ed.), 469 (1974).
7. Drapeau, G., "The primary structure of Staphylococcal protease." *Can. J. Biochem.*, **v. 56**, 534 (1978).
8. Dugas, H., Gaudet, F. and Leduc, P., "Structural studies of Staphylococcal protease. III. Binding of anions to the spin-labeled enzyme." *Can. J. Biochem.*, **v. 56**, 7 (1977).
9. Hall, T., et al., "Messenger RNA in G1 protein of french bean seed: Cell-free translation and product characterization." *Proc. Natl. Acad. Sci. USA*, **v. 75**, 3196 (1978).
10. Houmard, J., "Kinetic investigation of the Staphylococcal protease-catalyzed hydrolysis of synthetic substrates." *Eur. J. Biochem.*, **v. 68**, 621 (1976).
11. Houmard, J. and Drapeau, G., "Staphylococcal protease: A proteolytic enzyme specific for glutamoyl bonds." *Proc. Natl. Acad. Sci. USA*, **v. 69**, 3506 (1972).