Catalog Number: 102598, 102599, 195367

Pepsin

CAS # : 9001-75-6

Synonyms: Pepsin A; E.C. 3.4.23.1

Source: *Pig stomach mucosa*

Physical Description: A white to off-white freeze-dried powder

Unit Definitions:

Anson Unit: One unit will produce a ΔA_{280} of 0.001 per minute at pH 2.0 and 37°C, measured as TCA-soluble products using hemoglobin as substrate.

NF Unit: Pepsin digests not less than 3000 and not more than 3500 times its weight of coagulated egg albumin. (1 pepsin unit will digest 3000 units coagulated egg albumin at 52°C, pH 2-3, no time involved.)

Optimum pH: 1 to 3.5.[2]

Molecular Weight: Approximately 35,000.[1]

Extinction Coefficient: $E^{1\%}_{280} = 14.7$.[3]

Isoelectric Point: pH 1.0.[1]

Isoionic Point: 2.2-3.0.[4]

Inhibitors: Substrate-like epoxides[5], phenylacyl bromides[7], and diphenyldiazomethane.[8] Also inhibited by pepstatin A.

pH Stability: Unstable above pH 6.0.

Solubility: Freely soluble in water; dissolves readily in 0.01 M HCl (0.5 mg/ml - clear, colorless solution); practically insoluble in alcohol, chloroform or ether.

Description: Pepsin, an acid protease, contains a proteolytic enzyme. Pepsin contains the "cathepsin" component which has milk curdling activity. It has a broad range of substrate activity and demonstrates an esterase acitivity. It generally attacks peptide bonds.

NF Unit Assay (taken from the NF XII):

Mix 35 ml of 1.0 N hydrochloric acid with 385 ml of water. Dissolve 100 mg of pepsin in 150 ml of this dilute acid. Likewise dissolve 100 mg of NF Pepsin Reference Standard in another 150 ml portion of the dilute acid. Boil one or more hen eggs for 15 minutes to provide coagulated albumin for the assay. Cool them rapidly to room temperature by immersion in cold water; remove the shell and pellicle and all of the yolk and at once rub the albumin through a clean, dry No. 40 sieve, rejecting the first protion that passes through the sieve. Place 10 g of the succeeding well-mixed portion in each of 3 wide-mouth bottles of about 100 ml capacity. Immediately add 35 ml of the dilute acid at one time or in portions and, by suitable means, thoroughly disintegrate the particles of albumin. Place the bottles in a water bath at 52°C. After the contents of the bottles have reached that temperature, add 5.0 ml of the acidified solution of pepsin to one bottle, 4.30 ml of the same solution and 0.70 ml of the dilute acid to another bottle, and 5.0 ml of acidified solution of NF Pepsin Reference Standard to the third bottle. At once stopper the bottles securely, invert them 3 times, and maintain them at 52°C for 2 hours and 30 minutes, agitating the contents equally every 10 minutes by inverting the bottles once. Remove the bottles from the bath, pour the contents into 100 ml conically shaped measuring vessels, having diameters not exceeding 1 cm at the bottom and complying in other respects with the water and sediment tube ASTM Standard Method D96-35, graduated from 0 to 0.5 ml in 0.05 ml graduations; from 0.5 to 3 ml in 0.1 ml graduations; from 3 to 5 ml in 0.5 ml graduations; from 5 to 10 ml in 1 ml graduations; from 10 to 25 ml in 5 ml graduations; and with graduation marks at 50-, 75-, and 100 ml points. Transfer the undigested egg albumin which adheres to the sides of the bottles to the respective measuring vessels with the aid of small portions of water until 50 ml has been used for each. Mix the contents of each measuring vessel and allow them to stand for 30 minutes. The volume of the undissolved albumin in the measuring vessel corresponding to 5.0 ml of the solution of pepsin being assayed does not exceed the volume of the undissolved albumin in the measuring vessel corresponding to 5.0 ml of the solution of NF Pepsin Reference Standard, and the volume of the undissolved albumin in the measuring vessel corresponding to 4.30 ml of the solution of Pepsin being assayed is not less than the volume fo the undissolved albumin in the measuring vessel corresponding to 5.0 ml of the solution of NF Pepsin Reference Standard.

Note: Other measuring vessels than the one described in this monograph may be used if they are of such design and graduation as to measure the residue accurately.

Anson Unit Activity Assay:[6]

Unit Definition: One unit will produce a ΔA_{280} of 0.001 per minute at pH 2.0 and 37°C, measured as TCA-soluble products using hemoglobin as substrate.

Reagents:

A. 1.0 N HCl
B. 0.3 N HCl
C. 0.01 N HCl
D. 2.5% w/v Hemoglobin: Prepare by dissolving 2.5 grams bovine erythrocyte hemoglobin powder in 100 ml reagent grade water. Blend in a blender at maximum speed for 3-5 minutes. Filter through glass wool. Dilute 80 ml of filtrate with 20 ml of 0.3 N HCl (Reagent B).
E. 5% w/v Trichloroacetic acid (TCA)

Enzyme:

Dissolve pepsin at a concentration of 0.5 mg/ml in 0.01 N HCl. Keep chilled. Immediately prior to assay, dilute further in 0.01 N HCl to 5-20 micrograms per ml. Three dilutions are recommended.

Procedure:

Into each of six numbered test tubes pipette 5.0 ml hemoglobin substrate (Reagent D). Place in a 37°C water bath to equilibrate. Tubes 1-3 are blanks. Into each, pipette 10 ml of TCA (Reagent E) followed by 1 ml of enzyme dilution. Remove from bath after 5 minutes and filter. Read A_{280} of clear filtrate.

Tubes 4-6 are for test. At time intervals, add 1 ml of enzyme dilution to each and incubate at 37°C for exactly 10 minutes, stop the reaction by adding 10 ml of 5% TCA at timed intervals. Remove from bath after 5 minutes and filter. The filtrates should be clear. Record filtrate absorbance at 280 nm and subtract A_{280} of appropriate blank.

Calculation:

$$\text{Units/mg} = \frac{[A_{280}(\text{Filtrate}) - A_{280}(\text{Blank})] \times 1000}{10 \text{ minutes} \times \text{mg enzyme in reaction mixture}}$$

The above unit can be expressed as micromoles of tyrosine equivalents released per minute by multiplying by 16/1250 where 16 represents the final filtrate volume and 1250 is the extinction coefficient of tyrosine.

Availability:

Catalog Number	Description	Size
195367	Pepsin, activity approximately 2000 to 2400 Anson units/mg protein	250 mg 1 g 5 g 10 g
102598	Pepsin, 1:10,000; 3X NF standards	50 g 100 g 500 g 1 kg
102599	Pepsin, 1:15,000; 5X NF standards	25 g 100 g 250 g

References:

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2. Cornish-Bowden, A. and Knowles, J., "The pH-Dependence of Pepsin-Catalyzed Reactions." *Biochem. J.*, **v. 113**, 353 (1969).
3. Knowles, J., Sharp, H. and Greenwell, P., "The pH-Dependence of the Binding of Competitive Inhibitors to Pepsin," *Biochem. J.*, **v. 113**, 343 (1969).
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6. Anson, M., "The Estimation of Pepsin, Trypsin, Papain and Cathepsin with Hemoglobin." *J. Gen. Physiol.*, **v. 22**, 79 (1938).
7. Erlanger, B.F., et al., "Specific and Reversible Inactivation of Pepsin." *J. Biol. Chem.*, **v. 240**, PC3447 (1965).
8. Delpierre, G.R. and Fruton, J.S., "Inactivation of Pepsin by Diphenyldiazomethane." *Proc. Nat'l. Acad. Sci.*, **v. 54**, 1161 (1965).
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