Product Data Sheet

Pepsin, Porcine

½ **Cat#**	Pep16-100	Pepsin (>10,000 U/g), Porcine (low endotoxin, salmonella, and bacteria; industrial grade)	**Size**: 100 g
½ **Cat#**	Pep16-1000	Pepsin (>10,000 U/g), Porcine (low endotoxin, salmonella, and bacteria; industrial grade)	**Size**: 1 Kg

Pepsin is an enzyme (3.4.23.1) whose zymogen (pepsinogen) is released by the chief cells in the stomach and that degrades food proteins into peptides. It was the first animal enzyme to be discovered, and, in 1929, it became one of the first enzymes to be crystallized, by John H. Northrop. Pepsin is a digestive protease, a member of the aspartate protease family.

Pepsin is one of three principal protein-degrading, or proteolytic, enzymes in the digestive system, the other two being chymotrypsin and trypsin. The three enzymes were among the first to be isolated in crystalline form. During the process of digestion, these enzymes, each of which is specialized in severing links between particular types of amino acids, collaborate to break down dietary proteins into their components, i.e., peptides and amino acids, which can be readily absorbed by the intestinal lining. Pepsin is most efficient in cleaving peptide bonds between hydrophobic and preferably aromatic amino acids such as phenylalanine, tryptophan, and tyrosine.

Pepsin is an endopeptidase, which preferentially hydrolyzes those peptide linkages which involve the amino group contributed by the aromatic amino acids phenylalanine, tyrosine and tryptophan. Although pepsin digests proteins mainly into polypeptides of varying length, some shorter peptides and even some free amino acids, notably tyrosine and phenylalanine, may be released. Pepsin is most active in acidic environments between 37°C and 42°C. Accordingly, its primary site of synthesis and activity is the stomach (pH 1.5 to 2). Pepsin exhibits maximal activity at pH 2.0 and is inactive at pH 6.5 and above, however pepsin is not fully denatured or irreversibly inactivated until pH 8.0. Therefore pepsin in solution of up to pH 8.0 can be reactivated upon re-acidification. The stability of pepsin at high pH has significant implications on disease attributed to laryngopharyngeal reflux. Pepsin remains in the larynx following a gastric reflux event. At the mean pH of the laryngopharynx (pH = 6.8) pepsin would be inactive but could be reactivated upon subsequent acid reflux events resulting in damage to local tissues. Pepsin may also cause mucosal damage during weakly acidic or non-acid gastric reflux. Weak or non-acid reflux is correlated with reflux symptoms and mucosal injury. Pepsin in airway specimens is considered to be a sensitive and specific marker for laryngopharyngeal reflux. Pepsin from porcine stomach mucosa has been studied most extensively and has a molecular weight of 35 Kda.

Form and Storage

Pepsin is isolated from porcine stomach. Protein content is 95%. Enzyme activity of the lot is ~10,000 U/g). The powdered enzyme should be stored in the freezer (−20°C). If properly stored, these products have a shelf life of at least two years.

Solubility: Soluble in distilled water or dilute buffer

Unit Definition

The amount of enzyme which renders TCA soluble 0.001 E280 nm per/min at 37°C, using a denatured hemoglobin substrate.

QC testing

Aflatoxin:	<0.05 MPN/100g
Salmonella:	Zero in 10 g
Heavy Metals Lb	<5 mg/Kg
Heavy Metals Pb	<30 mg/Kg
Heavy Metals As	<3 mg/Kg
Bacteria	<100,000/g
Total Coliform	0 in 10 g

Assay Methods

Pepsin cleaves peptides from hemoglobin which are soluble in trichloroacetic acid (TCA). The tyrosine and tryptophan content of these TCA-soluble peptides is determined by the measurement of the extinction at 280 nm.

Reagents

1.0 N HCl.0.3 N HCl.; 0.01 N HCl; 2.0% (w/v) Hemoglobin;

5% (w/v) Trichloroacetic acid (TCA); Pepsin (enzyme) solution. Dissolve to a concentration of 0.5 mg/ml in 0.01 N HCl. Just prior to assay dilute further in 0.01 N HCl to a concentration of 5-20 micrograms per ml.

Procedure

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 280 nm and 37°C. Prepare water bath at 37°C.

2 Into each of 6 numbered test tubes pipette 5.0 ml hemoglobin substrate. Place all tubes in water bath at

37°C to equilibrate. Use tubes 1-3 as blanks; pipette 10 ml TCA, followed by 1 ml of diluted enzyme, into each of the blank tubes.

3 Remove tubes 1-3 from water bath and clarify.

Read E280 nm of clear filtrate.

4. Tubes 4-6, remaining in water bath, are for the enzyme test. Add 1 ml of the diluted enzyme to each of these tubes at timed intervals for exactly 10 min.

5 Stop the reaction by adding 10 ml of 5% TCA at timed intervals.

6 Remove tubes 4-6 from water bath after 5 min. and clarify (filtrates should be clear). Read E280 nm of filtrate and subtract E280 nm of the appropriate blank.

References Dun BM (2001) Curr Protoc Protein Sci Chapter 21: Unit 21.3; Johnston N 92004) Laryngoscope 114 (12): 2129–34; Knight J (2005) Laryngoscope 115 (8): 1473–8;

For in vitro research use only

Related Material available for ADI

Catalase, Peroxidase, Glucose Oxidases

PEP16-100-Pepsin-Porcine 150817A

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