

Technical Data Sheet

Pepsin 1:10000 NF12

for microbiology

Order number: 1403

Pepsin is a protein-degrading enzyme that is formed from an inactive precursor, pepsinogen, in the stomach under the hydrolytic effect of the body's own hydrochloric acid. Pepsin is an acidic endopeptidase with a molecular weight of about 36 kDa. The maximum activity of pepsin - in accordance with its site of synthesis and natural site of action - is in the highly acidic at pH values of 1-3. Above pH 4, the function of the enzyme decreases significantly and at pH values above 6.5, no activity at all is to be expected. The denaturation process caused by changes in pH values is reversible up to pH 8; if pepsin is exposed to even higher pH values, acid-induced reactivation of the protein is no longer possible.

Pepsin tolerates the presence of high concentrations of urea and guanidine hydrochloride and stays active at temperatures up to 60°C. The optimum working range of pepsin is 37 - 42 °C.

Pepsin cleaves proteins preferentially at the carboxyl groups (hence N-terminal) of aromatic amino acids such as phenylalanine and tyrosine. It does not cleave at bonds containing valine, alanine or glycine.

Pepsin is extracted from the stomach lining of pigs, cattle or sheep for industrial (food manufacturing) and biochemical applications. The origin of Pepsin 1:10000 for microbiology is the porcine mucosa.

Application

Pepsin is used for enzymatic digest of proteins in numerous biochemical and biotechnological applications. Some examples are:

- x treatment of proteins prior to mass spectrometric analyses and further applications in protein research.
- x digestion of antibodies (pepsin is commonly used in the preparation of F(ab')₂ fragments from antibodies).
- x investigation of disulfide bridging (since digestion is performed at low pH, the risk of disulfide exchange reactions is low.)

For reconstitution of the powdered enzyme, we recommend to use 10 mM HCl as a solvent. Stock solutions are commonly prepared with a concentration of 0.5 – 10 mg Pepsin/ml. Store solutions aliquoted at -20°C for up to 6 months. Short time storage is possible at 4°C.



Pepsin activity

Pepsin activity is expressed in many different units based on quite different methods of determination. Accordingly, it can be difficult or impossible to compare pepsins from different sources.

Please note: Even if the activity is generally noted by all manufacturers - for many applications the specific pepsin activity is not crucial.

The "1:10000" in the product description is a measure of the activity of the pepsin; it represents the ratio of enzyme to substrate: One pepsin unit will digest 10,000 units of coagulated egg albumin at 52°C, pH 2-3.

FCC and NF also define pepsin activity by its egg albumin digesting power:

FCC (Food Chemical Codex): *One pepsin unit is defined as that quantity of enzyme that digests 3000 times its weight of coagulated egg albumen under the conditions of the assay.*

NF (National Formulary): *Pepsin, when assayed as herein directed digests not less than 3000 and not more than 3500 times its weight of coagulated egg albumen.*

The FIP (International Pharmaceutical Federation), US Pharmacopoeia and European Pharmacopoeia use another approach for activity determination: They use hemoglobin as a substrate in a spectrophotometric assay.

EP, USP: not less than 0.5 units per milligram, dried basis.

Pepsin activity is also sometimes given in Anson units based on the method of Anson J. Gen. Physiol., 22, 79 (1938): *One unit will produce ΔA at 280 nm of 0.001 per minute at pH 2.0 and 37 °C, measured as TCA soluble products using hemoglobin as a substrate.*

It is possible to convert activities by using experimentally determined factors: 1 FIP unit/mg is equivalent to 6000 FCC units/mg. Accordingly, the following correlations can be made:

NF 3000 = 3000 FCC units/mg = 1:3000

and corresponds approximately to 1x USP (0.5 USP units/mg) = 0.5 EP units/mg = 0.5 FIP units/mg

NF 10000 = NF 1:10000 = 10000 FCC units/mg = 1:10000

and corresponds approximately to 3-4x USP = ~ 2 EP units/mg = 2 FIP units/g

= 2000 EP units/g = 2000 FIP units/g

Storage and reconstitution

Store pepsin powder at 2 to 8 °C. Make sure there is no contact with moisture. When stored under proper conditions, Pepsin powder remains stable for years.

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