
Princeton Separations, Inc.

Modified PEPSIN, Porcine Sequencing Grade

Cat. No. EN-180
5 x 20 μ g vials

Princeton Separations' Sequencing Grade endopeptidases are highly purified enzymes that are modified chemically to reduce autolysis and increase enzyme stability. Use of modified enzymes results in greater control of protein fragmentation reactions due to consistent enzyme activity over long digestion periods.

Characteristics

Porcine Pepsin is a serine endopeptidase with MW of 35KD. Its optimum activity is in the acid range (pH 2.0 - pH 4.0) and it predominantly cleaves peptide bonds on the carboxy side of aromatic and hydrophobic residues. However, it exhibits no hydrolytic activity if the adjacent amino acids are: Valine, Alanine or Glycine. The fact that Pepsin has proteolytic activity in the acid pH range, makes this enzyme a useful reagent for the fragmentation of proteins which are soluble exclusively in acid medium.

Chemical Modification

After extensive purification, the enzyme is stabilized by chemical treatment to reduce catalytic autolysis under reaction conditions. Thus, after incubation of the enzyme in 10mM HCl at 30°C for 4 hours and 24 hours, the remaining activity of the enzyme is in excess of 80% and 70%, respectively.

Meeting the Challenges of Proteomics



PRINCETON
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Quality Control

The modified Pepsin activity is monitored by a protease assay using bovine hemoglobin as substrate. In addition, the process of fragmentation of a standard protein (lysozyme) is also monitored. At a ratio of 1:50 and after incubation in 10 mM HCl for 4 hours (and 24 hours) at 30°C, four distinct peptides are always detected.

Preparation for Use

Sequencing Grade Modified Pepsin is supplied lyophilized in vials of 20µg. Reconstitute each vial with a minimum of 40µL of deionized water to obtain a final protein concentration of 0.5µg/µL in 50mM Ammonium Acetate, pH 4.5.

Application

For the fragmentation of acid soluble proteins, Pepsin is added to the protein to be digested at a ratio of 1:50 enzyme to substrate by mass. The digestion mixture is incubated at 30°C for 4 hours or overnight. In case the presence of urea can improve the digestion process, this reagent may be added at 1M or 2M concentration. The enzyme activity will not be affected.

Storage

Store unopened vials at 2°C - 8°C. Upon reconstitution in deionized water, vials may be stored at -10°C to -20°C for up to six months. Freeze thaw for up to 5-8 times does not affect the enzymatic activity.

Future Proteomic Products and Additional Information

In addition to sequencing grade, modified Pepsin, Trypsin, Arginine-C, Lysine-C, Glutamic-C, Aspartic-N and Chymotrypsin, Princeton Separations will continue to develop products in the proteomics area.

All future enzymes will be similarly modified to reduce autolysis, improve stability and simplify protein sequencing experiments. Smaller amounts of enzyme can be used over longer incubation times due to stability of the modified endoproteinases.

For information about Princeton Separations' current and new products, please visit our website at www.prinsep.com or email us at proteomics@prinsep.com.

