Meeting the Challenges of Proteomics

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79-101053A

Princeton Separations, Inc.

Immobilized PEPSIN (Porcine) Sequencing Grade, Modified 200µL Gel

Cat. No. EN-181

Characteristics

Porcine Pepsin is a serine endopeptidase with MW of 35KD. Its optimum activity is in the acid range (pH 2.0 - pH 3.0) 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. Pepsin is also widely used for Deuterium-hydrogen exchange.

Immobilization

Princeton Separations' Sequencing Grade Bovine Pepsin is immobilized on 0.2µ silica gel beads by covalent chemical bonds. The resulting gel is suspended in deionized water at 30% - 50% solids. After use in a protein disgestion reaction, the enzyme can be separated and completely removed from the digestion products by a short spin in a microfuge. As a result, the enzyme can be used in large excess, thus speeding up the rate of the catalytic reaction. At a ratio of 1:10, enzyme to protein substrate, a complete digestion of most proteins can be achieved in 30 to 60 minutes.

Quality Control

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

Preparation for Use and Application

Sequencing Grade Immobilized Bovine Pepsin is supplied as a gel in aqueous suspension and can be used directly from the container without any prior preparations such as washing or reconstitution. The composition of the Immobilized Pepsin gel is adjusted so that the activity of 1μL of gel suspension is equivalent to 0.5μg of native Pepsin.

Before the first use, centrifuge the vial for one (1) minute at 1000 - 2000 RPM to collect the reagent at the bottom of the vial. Always mix with the pipette before withdrawing a sample.

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 or 1:10 enzyme to substrate if fast reaction is desired. The digestion mixture is incubated at 30°C overnight or one (1) hour in the case of 1:10 conditions. For hard to digest protein, the presence of urea can help the digestion process. In such a case, the reagent may be added at 1M or 2M concentration. The enzyme activity will not be affected. The recommended buffer is 0.01M Hydrochloric Acid.

Storage

Store Immobilized Pepsin at 2°C - 8°C. DO NOT FREEZE. The product is stable for 1 year at 2°C - 8°C. Avoid microbial contamination by using sterile pipette tips when transferring the gel.

Future Proteomic Products and Additional Information

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