

KRISHGEN BioSystems

Modified Proteinases

Modified Chymotrypsin

Characteristics

It is a serine endopeptidase which predominantly cleaves peptide bonds on the carboxy side of Tyrosine, Phenylalanine and Tryptophan. In addition, Chymotrypsin also catalyzes the hydrolysis of peptide bonds at the carboxy side of Leucine, Methionine, Alanine, Aspartic and Glutamic acids, although at a much lower rate.

Application

For fragmentation of protein, Modified Chymotrypsin is added to the protein at a ratio of 1/50 to 1/200, by weight, in a standard digestion buffer such as 50 mM Tris HCl, pH 8.0 in 1mM CaCl₂. It is highly recommended to choose a ratio of enzyme to protein to allow for the shortest incubation time possible. This will reduce or eliminate the catalyzed hydrolysis of peptide bonds with non-aromatic amino acid residues.



Modified Trypsin Characteristics

Trypsin is a serine endopeptidase which specifically cleaves peptide bonds on the carboxy side of Arginine, Lysine and s-aminoethyl cysteine residues

Application

For protein fragmentation, modified Trypsin is typically added to the protein at a ratio of 1/20 to 1/100 (enzyme to protein, by weight) in a standard digestion buffer such as 50mM TrisHCl, pH 8.0. Incubation time will depend on the nature of the protein to be digested. The enzyme is stable for at least 24 hours at 30°C .

Modified Arginine-C Characteristics

It is a sulfhydryl endopeptidase which specifically hydrolyzes the carboxy peptide bond of Arginine. It is produced in cultures of the bacterium *Clostridium histolyticum* as an extra cellular enzyme. Arginine-C is susceptible to inactivation by oxidation and as a consequence requires reducing agents for protection. The enzyme also requires calcium ions for maximal activity.

Application

For protein fragmentation, it is typically added to the protein at a weight to weight ratio of 1/20 to 1/100 enzyme to protein

Modified Pepsin Characteristics

Porcine Pepsin is a serine endopeptidase with a molecular weight of 35 kD. Pepsin predominantly cleaves peptide bonds on the carboxy side of aromatic and hydrophobic residues.

Application

For the fragmentation of acid soluble proteins, Pepsin is used at a ratio of 1:50 enzyme to substrate by mass. The digestion mixture can be 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.

Modified Glutamic-C Characteristics

Glutamic-C endopeptidase from *S. aureus* V8 is a serine protease highly specific for the cleavage of peptide bonds at the carboxy side of either Aspartic or Glutamic acid.

Application

For protein fragmentation, it is typically added to the protein at a ratio of 1/20 to 1/100 enzyme to protein, by weight, in a standard digestion buffer such as 50mM Tris HCl, pH 8.0. An optimum time for incubation can be obtained by adjusting the enzyme to sample ratio. Glutamic-C endopeptidase from *S. aureus* V8 is as active as Trypsin on a mass basis.

Modified Aspartic-N Characteristics

It is a metallo enzyme isolated from a mutant of *Pseudomonas fragi*. The enzyme specifically hydrolyzes peptide bonds on the N-terminal side of aspartic and cysteic acids. The enzyme has a molecular weight of 27 kD.

Application

For protein fragmentation the enzyme is added to the protein to be digested at a ratio of 1/50 to 1/100, by weight in a standard digestion buffer pH 8.0. At overnight incubation a ratio of 1/100, enzyme to protein is adequate for most proteins.

Modified Lysine-C Characteristics

Lysine-C endopeptidase from *Lysobacter enzymogenes* is a serine protease with high specificity for the cleavage of peptide bonds on the carboxy side of Lysine.

Application

For protein fragmentation, modified Lysine-C is typically added to the protein at a ratio of 1/20 to 1/100 enzyme to protein, by weight, in a standard digestion buffer, pH 8.0. An optimum time for incubation can be obtained by adjusting the enzyme to sample ratio, taking into consideration that Lysine-C digestion activity is typically only 40% that of Trypsin.