

Modified Trypsin

Sequencing Grade

Cat. No. EN-110

4 x 25µg vials

Highly Purified
Extremely Low Autolysis
Increased Enzyme Stability
Greater Control of Protein Fragmentation
Consistent Enzyme Activity over Long Digestion Periods

Characteristics

Trypsin is a serine endopeptidase which specifically cleaves peptide bonds on the carboxy side of Arginine, Lysine and s-aminoethyl cysteine residues. There is little or no cleavage at arginyl-proline or lysyl-proline bonds. Our trypsin is obtained from Bovine pancreas certified to originate in the United States.

Chemical Modification

Princeton Separations' sequencing grade trypsin is subjected to extensive purification to remove contaminating proteases and tryptic autolysis by-products which could affect the specificity of the digestion process. Highly purified enzyme is modified chemically by a process developed at Princeton Separations. As a result, the modified enzyme is more resistant to autolysis and has improved stability. The modified enzyme retains at least 90% of its activity after 6 hours' incubation at 30°C in reaction buffer and at least 70% of its activity after 24 hours of incubation under the same conditions with retention of specificity.

Quality Control

Our Sequencing Grade Trypsin is characterized by assays which relate to its use in sequencing applications. Two assays are used for Quality Control: an amidase assay and a protein (digestion) assay using casein as substrate. The activity against casein is routinely compared with unmodified Trypsin and a Trypsin Activity Equivalence is calculated. To check for enzyme specificity an array of synthetic peptides is used.

Preparation

Modified Trypsin, Sequencing Grade, is supplied lyophilized in vials of 25µg. Reconstitute to a concentration of 1.0µg/µL in deionized water (25 µL per vial). The reconstituted enzyme is stable at 2-8°C for two to four days.

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 (NH₄)HCO₃, pH 7.8-8.0. The incubation is allowed to proceed at 25-30°C for 1 to 10 hours, but can extend to 24 hours in some applications. Incubation time will depend on the nature of the protein to be digested. The Princeton Separations enzyme is stable for at least 24 hours at 30°C and the autodigestion products are minimal. However, it is always preferable to use the shortest incubation time possible, since cleavage, particularly on the carboxy side of hydrophobic residues, has been found to occur following prolonged incubations (>8 hours). Optimal incubation time can be obtained by adjusting the enzyme to sample ratio.

Storage

The unused portion of the reconstituted enzyme should be frozen at -20°C.

Stability in Presence of Denaturing Agents

In the event that proteins are difficult to solubilize, denaturing agents such as urea or guanidine HCl may be added to the protein mix prior to digestion. The following table contains data on the effect of these agents on the enzymatic activity of the modified Trypsin.

<u>Denaturing Agent</u>	<u>Concentration</u>	<u>% Activity of Control</u>
Control	none	100%
Urea	0.1M	100%
Urea	0.5M	100%
Urea	1.0M	100%
Guanidine-HCl	0.05M	80%
Guanidine-HCl	0.1M	70%
Guanidine-HCl	0.25M	50%
Guanidine-HCl	0.50M	0%

References

Gary E. Means and Robert E. Feeney. *Biochemistry* 7, 1968

Princeton Separations' Sequencing Grade Endopeptidases

	Catalog No.	Size	Price (EURO)
Modified Trypsin, Sequencing Grade	EN-110	4 x 25µg	80,00
Modified Arginine-C, Sequencing Grade	EN-120	3 x 5µg	415,00
Modified Lysine-C, Sequencing Grade	EN-130	3 x 5µg	415,00
Modified Glutamic-C, Sequencing Grade	EN-140	4 x 10µg	145,00