

Modified Trypsin (Porcine)

Sequencing Grade

Cat. No. EN-151

**5 x 20 µg Vials Modified Porcine Trypsin +
1 x 10 µg Vial Autolyzed Porcine Trypsin Control**

A quality product from Princeton Separations, Inc.

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

Characteristics

Trypsin is a serine endoproteinase 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.

Chemical Modification

Princeton Separations' Sequencing Grade Modified Trypsin is first treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) and then subjected to extensive purification to remove contaminating proteases and tryptic autolysis by-products which could affect the specificity of the digestion process. The 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. Thus, Sequencing Grade Modified Trypsin retains 70% to 90% of its activity after 6 hours incubation at 30°C in reaction buffer and 65% to 80% of its activity after 24 hours of incubation under the same conditions.

Quality Control

Princeton Separations' Sequencing Grade Modified 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 for Use

Sequencing Grade Modified Trypsin is supplied lyophilized in vials of 20 µg. Reconstitute each vial with a minimum 40 µL of deionized water to obtain a final concentration of 0.5 µg/µL. If required, one vial of Autolyzed Trypsin is included for internal calibration. Reconstitute the vial of autolyzed Trypsin in 100 µL deionized water (final concentration is 100 ng / 1.0 µL).

Application

For protein fragmentation, Sequencing Grade Modified Trypsin is typically added in an enzyme to protein ratio (w/w) of 1:20 to 1:100, in a standard digestion buffer such as 50mM Tris HCl, pH 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. Sequencing Grade Modified Trypsin is stable for at least 24 hours at 30°C and the auto-digestion 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

Store unopened vials at 2-8°C. The unused portion of the reconstituted enzyme can be stored up to one year frozen at -10°C to -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 Sequencing Grade 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%
Urea	2.0M	100%
Urea	4.0M	100%
Guanidine-HCl	0.05M	80%
Guanidine-HCl	0.1M	70%
Guanidine-HCl	0.25M	50%
Guanidine-HCl	0.5M	0%

References

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

Additional Sequencing Grade Endoproteinases by Princeton Separations:

Product	Size	Catalog No.
Modified Arginine-C, Sequencing Grade	3 x 5 µg	EN-120
Modified Lysine-C, Sequencing Grade	3 x 5 µg	EN-130
Modified Glutamic-C, Sequencing Grade	4 x 10 µg	EN-140
Modified Aspartic-N, Sequencing Grade	2 x 5 µg & 1 Activator Vial	EN-171
Modified Chymotrypsin (Bovine), Sequencing Grade	4 x 25 µg	EN-160
Modified Pepsin (Porcine), Sequencing Grade	5 x 20 µg	EN-180
Immobilized Pepsin (Porcine)	1 x 200 µL Gel	EN-181
Immobilized Trypsin (Porcine)	1 x 200 µL Gel	EN-251
Immobilized Chymotrypsin (Bovine)	1 x 200 µL Gel & 1 mL Buffer	EN-261