

# Modified Chymotrypsin (Bovine) Sequencing Grade

**Cat. No. EN-160**

**4 x 25µg vials**

**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**

Chymotrypsin is a serine endoproteinase which predominantly cleaves peptide bonds on the carboxy side of Tyrosine, Phenylalanine and Tryptophan. In addition, Chymotrypsin also catalyses the hydrolysis of peptide bonds at the carboxy side of Leucine, Methionine, Alanine, Aspartic and Glutamic acids, although at a much lower rate. It is therefore recommended to always use the shortest digestion time possible.

## **Chemical Modification**

Princeton Separations' Sequencing Grade Modified Chymotrypsin is first treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) and then subjected to an extensive purification process to remove contaminating protease and chymotryptic 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 autolytic inactivation and has improved stability. Thus, Sequencing Grade Modified Chymotrypsin retains in excess of 80% of its activity after six hours at 30°C in reaction buffer, and in excess of 70% of its activity under the same conditions after 24 hours.

## **Quality Control**

The activity of Sequencing Grade Modified Chymotrypsin is monitored by assays developed to specifically relate to its activity in sequencing applications. These assays, an amidase assay using Benzoyl Tyrosine para-Nitroanilide and a protease assay using casein as substrate, were developed to replace the unreliable BTEE assay (Benzoyl Tyrosine Ethyl Ester) which is, in fact, an esterase assay. Assay results are routinely compared with that of freshly prepared unmodified Chymotrypsin. Furthermore, the enzyme protein digestion activity is compared to Trypsin and a Trypsin Activity Equivalence is calculated and reported. To check for specificity, an array of synthetic peptides is used.

### Preparation for Use

Sequencing Grade Modified Chymotrypsin is supplied lyophilized in vials of 25 µg. Reconstitute the enzyme at a concentration of 1.0 µg/µl by addition of 25 µl of deionized water.

### Application

For fragmentation of protein, Sequencing Grade Modified Chymotrypsin is typically added in an enzyme to protein ratio (w/w) of 1:50 to 1:200, in a standard digestion buffer such as 50mM Tris HCl, pH 8.0 in 1mM CaCl<sub>2</sub>. The digestion mixture is allowed to incubate at 25°C-30°C for 1 to 10 hours, but can be extended to 24 hours. However, 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.

### Storage

Store unopened vials at 2-8°C. Upon reconstitution in deionized water, vials may be stored up to 6 months at -20°C.

### Stability in the Presence of Denaturing Agents

For difficult to solubilize proteins, denaturing agents such as Urea or Guanidine-HCl may be added to the digestion mix prior to the digestion process. The following table contains data on the effect of these agents on the enzymatic activity of Sequencing Grade Modified Chymotrypsin at various concentrations.

<u>Denaturing Agent</u>	<u>Concentration</u>	<u>% Activity of Control</u>
Control	None	100%
Urea*	0.5M	100%
Urea	1.0M	100%
Urea	2.0M	100%
Urea	3.0M	100%
Urea	4.0M	100%
Guanidine-HCl	0.05M	100%
Guanidine-HCl	0.1M	100%
Guanidine-HCl	0.25M	100%
Guanidine-HCl	0.5M	11%

\*Before adding the enzyme, the pH of the Urea or Guanidine-HCl should be adjusted to that of the digestion reaction.

### Additional Sequencing Grade Endoproteinases by Princeton Separations:

<b>Product</b>	<b>Size</b>	<b>Catalog No.</b>
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 Trypsin (Porcine), Sequencing Grade	5 x 20 µg	EN-151
Modified Aspartic-N, Sequencing Grade	2 x 5 µg & 1 Activator Vial	EN-171
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

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