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Princeton Separations, Inc.

Immobilized Bovine Chymotrypsin, 200µL Gel, Sequencing Grade Modified, Cat. EN-261

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 hydrolysis 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.

Immobilization

Princeton Separations' Sequencing Grade Immobilized Bovine Chymotrypsin, TLCK (Tosyl-L-Lysine Chloro Methyl Ketone) treated, 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 digestion 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
Princeton Separations' Sequencing Grade, Modified Immobilized Chymotrypsin is characterized by assays which relate to its use in sequencing applications. Two assays are used for Quality Control: an amidase assay, using Benzoyl Tyrosine para-nitroanilide and a protease assay using casein as substrate. These assays were developed to replace the unreliable BTEE assay (Benzoyl Tyrosine Ethyl Ester) which is, by contrast, an esterase assay. The results are routinely compared with freshly prepared native Chymotrypsin. Furthermore, the enzyme protein digestion activity is also compared to Trypsin activity; and according to these measurements, a Trypsin Activity Equivalence is calculated and reported. To check for enzyme specificity an array of synthetic peptides is used.

Preparation
Sequencing Grade, Modified Immobilized Bovine Chymotrypsin 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 Chymotrypsin Gel is adjusted so that 1μL of gel suspension is equivalent to 0.5 μg of native Chymotrypsin. A reaction buffer, 1M Triethylamine acetate, pH 8.0 is provided. This buffer is volatile and can be removed easily by Speed-Vac centrifuge. Ammonium bicarbonate at pH 8 can also be used; however the buffer capacity of Triethylamine acetate is better in the pH 8 range.

Stability in Presence of Urea
For difficult to solubilize proteins, denaturing agents such as urea may be needed in the protein mix prior to the digestion process. The Princeton Separations' Immobilized Chymotrypsin retains its full activity through the range 1M to 8M Urea. We do not recommend the use of guanidine HCl when using Chymotrypsin in any form.

Application
The Chymotrypsin gel suspension is ready to use. Before the first use centrifuge the vial for one minute at 1000 - 2000 RPM to collect the reagent at the bottom of the vial. Always mix with the pipette before withdrawing a sample.

The recommended buffers are 0.1 M Triethylamine acetate, pH 8 or 0.1 M Ammonium bicarbonate, pH 8. One mL of 1M Triethylamine acetate, pH 8.0 is provided. This buffer is highly volatile and can be easily removed by centrifugation in a Speed-Vac centrifuge. Incubation is best at 30°C - 37°C. At the end of the digestion period the reaction mix is centrifuged for two minutes in a microfuge at 8,000 - 10000 RPM to separate the enzyme from the digestion products. The supernatant (containing the products) is collected with a micropipette. To recover remaining traces of the product, 10μL deionized water are added to the precipitate, mixed, then centrifuged as described above.

For accelerated digestion (30 - 60 minutes), a ratio of 1:10 enzyme to protein substrate is recomended. For routine digestion (4 hours - overnight) the recommended ratio is 1:25.

Storage
Store unopened vials of Immobilized Chymotrypsin at 2-8°C. DO NOT FREEZE.
The product is stable for 6 months at 2-8°C. Avoid microbial contamination by using sterile pipette tips when transferring the gel.