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Meeting the Challenges of Proteomics

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Princeton Separations, Inc.

Immobilized Porcine Trypsin, 200µL Gel

Sequencing Grade, Modified

Cat. EN-251

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.

Immobilization

Princeton Separations' Sequencing Grade Immobilized Porcine Trypsin, TPCK treated, is immobilized on silica 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 Trypsin is characterized by assays which relate to its use in sequencing applications. Two assays are used for

Quality Control: an amidase assay (using Benzoyl-Arginyl-para-nitroanaline as substrate) 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. Each lot contains a Certificate of Analysis describing test results for the lot.

Preparation

Sequencing Grade, Modified Immobilized Porcine Trypsin 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 Trypsin Gel is adjusted so that 1μL of gel suspension is equivalent to 0.5 μg of native Trypsin. 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 Trypsin retains its full activity through the range 1M to 8M Urea. We do not recommend the use of guanidine HCl when using Trypsin in any form.

Application

The Trypsin 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. If a micropipette tip is used, it is recommended that the pipette tip be cut 1 - 2 mm from the tip to allow a larger orifice. The recommended buffers are 0.1M Triethylamine acetate, pH 8.0 or 0.1M Ammonium bicarbonate, pH 8.0. 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 30ºC - 37ºC. At the end of the digestion period the reaction mix is centrifuged for two minutes in a microfuge at 8,000 - 10,000 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 recommended. For routine digestion (4 hours - overnight) the recommended ratio is 1:25.

The Princeton Separation Immobilized Trypsin is especially helpful when a multiple enzymatic digestion for enhanced sequence coverage of protein peptides is planned. Using a combination of Trypsin and Glutamic-C (Biringer, et al.) were successful in increasing the coverage by more than 50% (1).

Storage

Store unopened vials of Immobilized Trypsin at 2-8ºC. DO NOT FREEZE.

The product is stable for 6 months at 2-8ºC. Avoid microbial contamination by using sterile pipette tips when transferring the gel.

References

(1) R. G. Biringer, F.M. Maroto, H. Amato, M.G. Harrington, A. F. Huhmer **Enhanced sequence Coverage of Proteins in Human Cerebrospinal Fluids, using multiple Enzymatic digestion and linear ion Trap LC-MS/MS.** Presented at the Poster Sessions of 2004 ABRF meeting in Austin, TX. Princeton Separations will send a reprint upon request.