INSTRUCTIONS

Immobilized TPCK Trypsin

20230

0479.3

Number	Description
20230	**Immobilized TPCK Trypsin,** 2mL of settled gel supplied as a 50% slurry containing glycerol and 0.05% sodium azide as a preservative
	Source: Bovine pancreas
	Gel Support: Crosslinked 4% beaded agarose
	Activity: ≥ 200 TAME units per mL of gel, where one unit is equal to 1 µmole of TAME (*p*-toluenesulfonyl-L-arginine methyl ester in the presence of Ca^{2+}) hydrolyzed/min at pH 8.2, 25°C (One TAME unit = 19.2 National Formulatory units = 57.5 BAEE units)
	Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

Trypsin immobilized on beaded agarose makes it possible to eliminate enzyme contamination of tryptic digests. The trypsin can be easily removed from the digest by separating the trypsin gel from the digestion solution. The Thermo Scientific Immobilized TPCK Trypsin is treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK), which is a reagent that has been reported to inhibit chymotrypsin activity without effect on trypsin.

Trypsin is a 23,200 molecular weight protein with a pH optimum between 7.5 and 9.0. The isoelectric points of trypsinogen and trypsin are 10.5 and 9.3, respectively. Trypsin has a wide range of applications including amino acid analysis and protein sequencing studies. Enzymes such as trypsin and chymotrypsin are selective in their cleavage of peptide bonds and have become important tools in sequencing studies. Chymotrypsin cleaves peptide bonds in which the carboxyl group is contributed by phenylalanine, tryptophan and tyrosine. In contrast, trypsin cleaves only those peptide bonds in which the carboxyl group is contributed by lysine or arginine residues, regardless of the length or amino acid sequence of the chain. The total number of resulting peptides can be estimated from the number of lysine and arginine residues in the protein. Ion-exchange chromatography, paper electrophoresis or peptide mapping can be used to separate digestion fragments.

Procedure for Trypsin Digestion

Note: The following protocol must be optimized for each specific application. Increasing the enzyme to substrate (E/S) ratio and incubation temperature increases the reaction rate.

1. Prepare a digestion buffer consisting of 0.1M NH_4HCO_3 buffer, pH 8.0.
2. Dissolve 1mg of the protein sample in 0.5mL digestion buffer.
3. Wash 0.10-0.25mL of the Immobilized TPCK Trypsin with 3 × 500µL of digestion buffer. Separate the gel from the buffer after each wash by centrifugation or by using a serum separator (Product No. 44886).
4. Suspend the gel with ~0.2mL of the digestion buffer.
5. Add the Immobilized TPCK Trypsin to the protein sample.
6. Incubate the reaction mixture in a rapidly shaking water bath for 2-18 hours at 37°C.
7. Separate the trypsin gel from the digestion mixture by centrifugation or by using a resin separator (Product No. 69710).

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Additional information

Example Applications

- **Protein Sequencing:** The peptides formed using trypsin can be sequenced by Edman Degradation, which labels and removes the amino terminal residue from the peptide while leaving all other peptide bonds intact. The newly exposed amino-terminal residue can subsequently be labeled and removed by repeating the same reaction series. By using this procedure, the entire sequence of a peptide can be determined.
- **Antibody Fragments:** Trypsin has been used to prepare $F(ab')_2$ fragments from sheep IgG, which is reported to be resistant to digestion by pepsin.

  Fab and Fc fragments from human and mouse IgM have been prepared in the presence of 5M urea. Mouse IgM was incubated with TPCK Trypsin at an enzyme to substrate ratio (E/S) of 1:100 in 50mM Tris•HCl (pH 8.0) containing 150mM NaCl and 20mM $CaCl_2$ for 5 hours at 37°C. Mercaptoethanol was then added to an adjusted final concentration of 10mM. The resulting product was incubated for 5 minutes at 37°C and 0.1mg/mL soybean trypsin inhibitor was added. After an additional 5 minute incubation, the solution was adjusted to 60mM iodoacetamide and incubated at room temperature for 10 minutes. Finally the sample was dialyzed against four changes of phosphate buffered saline.

  Trypsin digestion of several species of IgM was studied (see Reference section). The digestion was performed in 0.1M Tris•HCl, 0.2M NaCl, 0.01M $CaCl_2$, pH 8.3 at an E/S 1:100 (w/w) at 55°C. The digestion of mouse yielded $F(ab')_2$ and Fab but not $(Fc)_{5\mu}$. Perhaps, at 55°C, the $C\mu_3$ and the $C\mu_4$ domains of the mouse are unstable and the proteolytic sites are accessible. Trypsin digestion of human IgM yielded Fab and $(Fc)_5$. The $(Fc)_5$ was readily degraded to subunits and peptides.

Related Thermo Scientific Products

20233	**TPCK Trypsin,** 50mg
89895	**In-Solution Tryptic Digestion and Guanidination Kit**
89871	**In-Gel Tryptic Digestion Kit**
89870	**Pierce® C18 Spin Columns,** 25/pkg
28904	**Trifluoroacetic Acid, Sequanal Grade,** 10 × 1mL
88300	**Pierce Fe-NTA Phosphopeptide Enrichment Kit**

General References

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