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Product Information

Trypsin from porcine pancreas
Proteomics Grade, BioReagent, Dimethylated

Catalog Number **T6567**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

EC 3.4.21.4
CAS RN 9002-07-7

Product Description
Trypsin is routinely used in proteomics research for peptide mapping and protein sequence work, due to its highly specific cleavage resulting in a limited number of tryptic peptides.[1-5] Trypsin is a pancreatic serine endoprotease which hydrolyzes peptide bonds specifically at the carboxyl side of arginine and lysine residues. The rate of hydrolysis is slower if an acidic residue is on either side of the cleavage site and cleavage may not occur if a proline residue is on the carboxyl side.[1-5] The enzyme also exhibits esterase and amidase activities.[1] Trypsin has an average molecular mass of 23.29 kDa and a pH optimum near 8.0.[1]

Proteomics Grade Trypsin has been extensively purified from porcine pancreas. The lysine residues have been reductively methylated, producing a stable product that is resistant to autolysis.[6] It has also been TPCK treated to remove chymotryptic activity. The product is further purified by affinity chromatography and lyophilized from dilute acetic acid. This process yields a highly purified trypsin product that is suitable for proteomics work. The highly purified and chemically stabilized Proteomics Grade Trypsin gives excellent performance for use in either solution or in-gel tryptic digestions (see Figures 1 and 2).

Protein content is based on $E^{1\%}$ = 14.4 at 280 nm.[7]

Specific activity: ≥10,000 BAEE units per mg protein.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Reconstitute the lyophilized product in 1 mM HCl at the concentration appropriate for the application. These instructions are for reconstitution of a 20 µg vial. To reconstitute other sized vials, use proportionally more diluent.

For Solution Digests – Prepare the trypsin in 1 mM HCl at a concentration of 1 mg/ml (20 µl of 1 mM HCl for a 20 µg vial). This results in a solution containing 1 mg/ml trypsin, pH 3.0.

For In-gel Digests – Prepare a solution by adding 100 µl of 1 mM HCl to one 20 µg vial of trypsin. Mix the vial briefly to ensure the trypsin is dissolved. Add 900 µl of a 40 mM ammonium bicarbonate in 9% acetonitrile solution to the vial and mix. The final concentration of trypsin is 20 µg/ml [See Technical Bulletin for Trypsin Profile IGD Kit (Catalog Number PP0100) at our web site: www.sigmaaldrich.com/homepage.html].
Note: Alternately, one 20 µg vial of trypsin may be reconstituted with 100 µl of the 1 mM HCl and stored at 2–8 °C or at –20 °C. When ready to prepare the working trypsin solution, an aliquot of the acidic trypsin solution may be combined with the correct amount of the 40 mM ammonium bicarbonate in 9% acetonitrile solution (1 part of acidic trypsin solution to 9 parts of the 40 mM ammonium bicarbonate in 9% acetonitrile solution).

Storage/Stability
The lyophilized powder is stable for at least one year if stored unopened at 2–8 °C.

The acidic reconstituted solution (pH 3.0) can be stored at 2–8 °C for 2 weeks or at –20 °C for up to 4 weeks. The ammonium bicarbonate trypsin solution prepared for in-gel digests may be stored either at 2–8 °C for up to 2 weeks or as frozen aliquots for up to 4 weeks. Either trypsin solution is stable for at least 3 freeze-thaw cycles.

Procedures
The specificity and activity of trypsin is retained in systems containing up to 20% organic solvent.[8] In addition, trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% SDS.[2-5] The digestion pattern may be influenced by the buffer composition.[9] A peptide such as the insulin B chain, oxidized should be run as a control for all experiments.

A. Solution Digestion
For peptide or protein digestion, a ratio between 1:100 to 1:20 (w/w) of enzyme to substrate is recommended. Dissolve the peptide or protein substrate in 100 mM ammonium bicarbonate (Catalog Number A6141), pH 8.5, or 100 mM Tris-HCl, pH 8.5. The Tris-HCl buffer is incompatible with MALDI analysis and the ammonium bicarbonate buffer should be used when MALDI analysis will follow. Dissolve the trypsin in 1 mM HCl to a concentration of 1 mg/ml. Add the trypsin solution to the substrate protein solution. The recommended incubation time is between 2–18 hours at 37 °C depending on the enzyme to substrate ratio.

An example of the high specificity of the Proteomics Grade Trypsin was demonstrated with the described solution digestion procedure using insulin B chain, oxidized (Catalog Number I1764) as a substrate (see Figure 1).

The sequence of insulin B chain, oxidized is:

FVNQHLC$_{ox}$GSHLVEALYLVC$_{ox}$GER GFFYTPK A

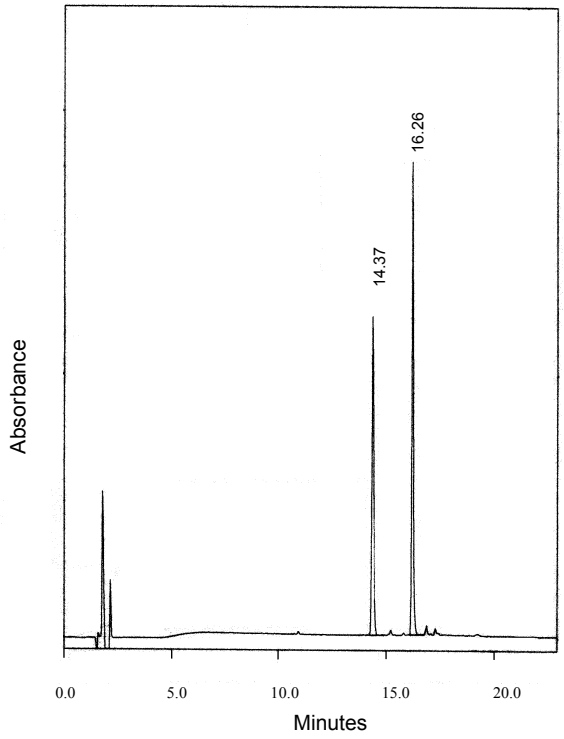
Tryptic Cleavage Sites

During the extensive 18 hour digestion only the expected peptides were generated with no indication of other proteolytic activity. The actual digestion time used can be determined by the user, as under the conditions used for the 18 hour digestion, the cleavage of the substrate peptide is complete in less than 5 minutes.

Retention Time (min)	Mass (Da)	Fragment
14.37	858.4	Gly(23)-Lys(29)
16.26	2583.0	Phe(1)-Arg(22)

The hydrophilic amino acid Ala (30) co-elutes with the buffer salts in the injection peak.

Figure 1.
Trypsin solution digestion of insulin B chain, oxidized.



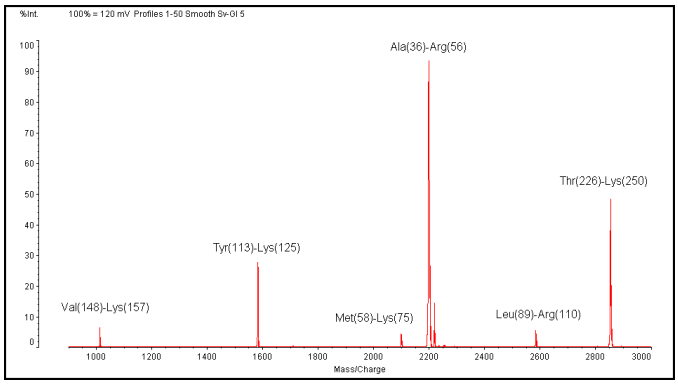
Insulin B chain, oxidized (100 µg) was digested with 2 µg of trypsin for 18 hours at 37 °C in 100 µl of 100 mM Tris-HCl, pH 8.5. A 10 µg aliquot was separated on a Supelco Discovery® C_{18} column (15 cm × 4.6 mm, 5 micron, Catalog Number 504955), using a 20 minute linear gradient from 5-50% B at 1 ml/min with UV detection at 214 nm and by mass spectrometry. Solvent A: 0.1% (v/v) TFA in water and Solvent B: 0.08% (v/v) TFA in acetonitrile.

B. In-gel Digestion[17]
Trypsin may also be used for in-gel protein digestions with subsequent identification by mass spectrometry. An example is shown in Figure 2. Literature has been published, which describes digestion protocols from gels or on membranes.[10-17] The following procedure starts with a Coomassie® Brilliant Blue, SYPRO® Orange, or SYPRO Ruby stained 1D or 2D polyacrylamide gel. For silver stained gels, a gel destaining step different than that used for dye stained gels is required. The ProteoSilver™ Plus Silver Staining Kit (Catalog Number PROTSIL2) is recommended for silver staining prior to tryptic digestion and MS analysis. It contains destaining solutions for silver stained gels and a procedure for preparing gel slices for tryptic digestion.

1. Carefully cut the band of interest from a 1D gel or the protein spot from a 2D gel, using a scalpel or razor blade, taking care to include only stained gel. Lift out the gel piece using clean flat nosed tweezers.
2. Place the gel piece in a siliconized Eppendorf® tube or equivalent. A siliconized tube reduces binding of the peptides to the tube surface. If unsure of chemicals leaching from the tube, which could interfere or suppress the MALDI-MS signal, prewash the tube with 100 µl of a 0.1% trifluoroacetic acid in 50% acetonitrile solution and then allow it to dry before use.
   Note: The gel piece may be cut into equal sections of 1–1.5 mm size and the sections may be used in place of the intact piece.
3. Cover the gel piece with 200 µl of 200 mM ammonium bicarbonate with 40% acetonitrile) and incubate at 37 °C for 30 minutes. Remove and discard the solution from the tube.
4. Repeat Step 3 one more time.
5. Dry the gel piece in a Speed Vac® for 15–30 minutes.
6. Add 20 µl (0.4 µg of trypsin) of the trypsin solution prepared for in-gel digests to the gel sample. (See Preparation Instructions.)
7. Add 50 µl of 40 mM ammonium bicarbonate in 9% acetonitrile solution to the gel sample.
8. Confirm that the gel piece is at the bottom of the tube and covered with liquid.
9. Incubate for 4 hours to overnight at 37 °C.
   Note: A shorter digestion time may be sufficient, but may yield slightly lower sequence coverage.
10. After the incubation, remove the liquid from the gel piece and transfer the liquid to a new labeled tube. This solution contains the extracted tryptic peptides. If MALDI analysis is to be performed at this step, acidification with TFA prior to matrix addition may be needed.
11. Add 50 µl of a 0.1% trifluoroacetic acid in 50% acetonitrile solution to the gel piece and incubate for 30 minutes at 37 °C.
    Note: This extraction step only increases the peptide yield by about 5%.[17] If the extra 5% is not required, the extraction step can be eliminated and the sample solution from Step 10 may then be analyzed.
12. Remove the 0.1% trifluoroacetic acid in 50% acetonitrile solution and combine with the liquid from Step 10.
13. The combined sample solution from Step 12 is ready for MALDI-MS analysis.

Note: If digesting low levels of protein, the sample mixture may need to be concentrated with a ZipTip® before spotting on the MALDI target.

Figure 2.
MALDI analysis of an in-gel digest of carbonic anhydrase II.



The substrate protein, carbonic anhydrase II (0.5 µg), was separated on a 4–20% Tris-Glycine gel. The spot was removed and digested using the protocol for in-gel digestion. The matrix (α-cyano-4-hydroxycinnamic acid) was prepared at 10 mg/ml in 70% acetonitrile with 0.1% TFA. The digest solution was desalted using a C_{18} ZipTip and 1.5 µl of the matrix solution was used to directly elute the peptides onto the MALDI target.

Note: A common autolytic fragment observed from a trypsin digest is 842.51 (A_7) m/z produced by arginine cleavage. Other autolytic peptides occasionally detected include the 2239.14 (A_4) and 1045.56 (A_6) m/z. The cited peptide at 2211.10 (A_4) m/z containing an unmodified Lys^{69} is not observed in the Proteomics Grade Trypsin, as it is fully converted to the dimethylated 2239.14 m/z peptide.

Related Products

Products Suitable for Protein Sequencing

Product Name	Catalog Number
α-Chymotrypsin	C6423
Endoproteinase Arg-C	P6056
Endoproteinase Asp-N	P3303
Endoproteinase Glu-C	P6181
Endoproteinase Lys-C	P3428
Leucine aminopeptidase	L9776
Insulin Chain B, Oxidized	I1764
α-Melanocyte Stimulating Hormone	M4135

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