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## Product Information

### Trypsin Proteomics Grade

Product Code **T 6567**  
Storage Temperature 2-8 °C

## TECHNICAL BULLETIN

E.C. # 3.4.21.4  
CAS# 9002-07-7

### Product Description

Trypsin is routinely used in proteomics for peptide mapping and protein sequence work, due to its highly specific cleavage resulting in a limited number of tryptic peptides.<sup>1-5</sup> 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.<sup>1-5</sup> The enzyme also exhibits esterase and amidase activities.<sup>1</sup> Trypsin has an average molecular weight of 23.29 kDa and a pH optimum near 8.0.<sup>1</sup>

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.<sup>6</sup> 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 as shown in Figures 1 and 2.

Protein content is based on  $E^{1\%} = 14.4$  at 280 nm.<sup>7</sup>  
The specific activity is minimum 10,000 BAEE units per mg protein.

### Precautions and Disclaimer

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

### Preparation Instructions

Reconstitute the lyophilized product in 1 mM HCl at the correct concentration for your application. For solution digests, prepare the trypsin in 1 mM HCl at a concentration of 1 mg/ml (20  $\mu$ l of 1 mM HCl for the 20  $\mu$ g vial). This results in a solution containing 1 mg/ml trypsin, pH 3.0.

For in-gel digests, prepare a solution by adding 100  $\mu$ l of 1 mM HCl to one vial of trypsin. Mix the vial briefly to ensure the trypsin is dissolved. Add 900  $\mu$ l of a 40 mM ammonium bicarbonate in 9% acetonitrile solution to the vial and mix. The final concentration of trypsin is 20  $\mu$ g/ml. (See Technical Bulletin for ProteoProfile™ Trypsin In-Gel Digestion Kit (Product Code PP0100) at our web site: [www.sigmaaldrich.com/homepage.html](http://www.sigmaaldrich.com/homepage.html).)  
Note: Alternately, one vial of trypsin may be reconstituted with 100  $\mu$ l 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

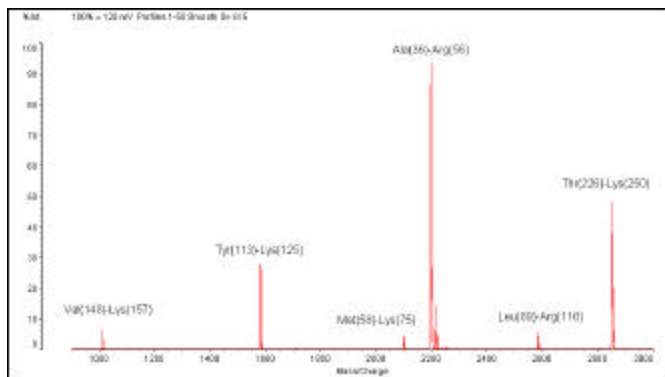
The lyophilized powder is stable for at least one year if stored unopened at 2-8 °C. The acidic reconstituted solution (pH 3.0) can be stored at 2-8 °C for 2 weeks or at -20 °C for up to 4 weeks. The ammonium bicarbonate trypsin solution prepared for in-gel digests may be stored either at 2-8 °C for 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.



- 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.
- 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 to 1.5 mm size and the sections may be used in place of the intact piece.
- 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.
- Repeat Step 3 one more time.
- Dry the gel piece in a Speed Vac® for approximately 15 to 30 minutes.
- Add 20 µl (0.4 µg of trypsin) of the trypsin solution prepared for in-gel digests to the gel sample. (See Preparation Instructions.)
- Add 50 µl of 40 mM ammonium bicarbonate in 9% acetonitrile solution to the gel sample.
- Confirm that the gel piece is at the bottom of the tube and covered with liquid.
- Incubate for 4 hours to overnight at 37 °C.  
Note: A shorter digestion time may be sufficient, but may yield slightly lower sequence coverage.
- 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.
- 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%.<sup>17</sup> If the extra 5% is not required for your system, the extraction step can be eliminated and the sample solution from Step 10 may then be analyzed.
- Remove the 0.1% trifluoroacetic acid in 50% acetonitrile solution and combine with the liquid from Step 10.
- 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 ( $\alpha$ -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<sub>18</sub> ZipTip and 1.5 µl of the matrix solution was used to directly elute the peptides onto the MALDI target. Data was acquired in linear positive ion mode with a Kratos compact discovery SEQ. The resultant mass spectrum is shown. A database search of the mass list was done using the Mascot database at a tolerance of 300 ppm. The protein was identified as carbonic anhydrase II with a MOWSE score of 102 and a sequence coverage of 42%. The tryptic peptides that were identified by the database are shown above.

Note: A common autolytic fragment observed from a trypsin digest is 842.51 (A<sub>7</sub>) m/z produced by arginine cleavage. Other autolytic peptides occasionally detected include the 2239.14 (A<sub>4</sub>) and 1045.56 (A<sub>6</sub>) m/z. The cited peptide at 2211.10 (A<sub>4</sub>) m/z containing an unmodified lysine<sub>69</sub> is not observed in the Proteomics Grade Trypsin, as it is fully converted to the dimethylated 2239.14 m/z peptide.

## Related Products

### SIGMA HPLC Purified Products:

Product Name	Product Code
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$\alpha$ -Chymotrypsin	C 6423
Endoproteinase Arg-C	P 6056
Endoproteinase Asp-N	P 3303
Endoproteinase Glu-C	P 6181
Endoproteinase Lys-C	P 3428
Leucine aminopeptidase	L 9776
Insulin Chain B, Oxidized	I 1764
Melittin	M 1407
Glucagon	G 7774
$\alpha$ -Melanocyte Stimulating Hormone	M 4135

### Titles of Related Books (Product Code):

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MICROCHARACTERIZATION OF PROTEINS (M 9807)  
NEW METHODS IN PEPTIDE MAPPING FOR THE CHARACTERIZATION OF PROTEINS (Z36,931-4)  
A PRACTICAL GUIDE TO PROTEIN AND PEPTIDE PURIFICATION FOR MICROSEQUENCING (Z35,034-6)  
SEQUENCING OF PROTEINS AND PEPTIDES: Laboratory Techniques in Biochemistry and Molecular Biology, 2nd Ed. (S 2521)  
PROTEOLYTIC ENZYMES: A Practical Approach (P 4926)  
PROTEIN STRUCTURE: A Practical Approach (Z37,548-9)  
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HPLC OF MACROMOLECULES: A Practical Approach (H 3409)  
PROTEIN SEQUENCING PROTOCOLS: Methods in Molecular Biology (Z37,395-8)

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