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Product Information

SOLu-Trypsin
recombinant, expressed in *Pichia pastoris*
Proteomics Grade

Catalog Number **EMS0004**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

CAS RN 9002-07-7
EC 3.4.21.4
Synonym: recombinant Trypsin, porcine sequence expressed in *Pichia pastoris*

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]

SOLu-Trypsin is prepared from recombinant trypsin, porcine sequence. It is naturally devoid of chymotryptic activity. This high quality trypsin is suitable for proteomics use and is supplied in a ready to use solution.

SOLu-Trypsin is formulated at 1 mg/mL to fit seamlessly into current proteomic workflows. Protein content is verified by Biuret analysis.

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 Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

SOLu-Trypsin is ready to use at 1 mg/mL. Centrifuge vial briefly to collect liquid in bottom before removing cap. Store unused portion in the cooler for up to 2 years.

The product may be diluted into buffer of choice before use. Long term stability may be compromised depending on buffer so it is best to prepare only the amount required for daily use.

Storage/Stability

The product is stable for at least 2 years at 2–8 °C.

Procedures

<u>In-solution Protein Digestion (50–100 μg protein)</u>
If using a different amount of protein, scale reagents accordingly.

1. If protein is in solution, dry by vacuum centrifugation.
2. Prepare a denaturing solution by combining 1 volume of 60 mg/mL sodium deoxycholate in methanol solution with 1 volume of 20 mM DTT or TCEP in 50 mM ammonium bicarbonate solution.
   a. Other buffers can be used such as 50 mM Tris, pH 8.5.
   b. Other denaturants may be used such as 40% trifluoroethanol.
3. Add 20 μL of the denaturing solution to the dried sample.
4. Incubate at 57 °C for 1 hour.
5. Alkylate the reduced sample by adding 5 μL of 200 mM iodoacetamide in 50 mM ammonium bicarbonate solution.
6. Incubate for 1 hour in the dark at room temperature.
7. Add 220 μL of 50 mM ammonium bicarbonate or Tris solution to dilute the methanol and denaturants.

8. Add SOLu-Trypsin solution (as provided) for an enzyme to protein ratio of 1:20 by mass.
9. Incubate at 37 °C for 4 hours to overnight.
10. Quench the digestion and precipitate the deoxycholate, if used, by adding 2 µL of formic acid. Vortex well and centrifuge to pellet the precipitate.
11. Transfer the clear supernatant to a clean tube.
12. Dry by vacuum centrifugation.
13. Reconstitute in 0.1% formic acid for LC-MS applications.

Notes: Denaturants other than sodium deoxycholate may be used. For example, 40% trifluoroethanol or acetone with or without 6 M urea are effective denaturants. Dilute with at least 3 volumes ammonium bicarbonate or Tris before adding trypsin.

Reduction and alkylation are optional but improve digestion efficiency. ProteoPrep® Reduction and Alkylation Kit (Catalog Number PROTRA) may also be used.

In-Gel Digestion

Trypsin may also be used for in-gel protein digestions with subsequent identification by mass spectrometry. 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. 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 tweezers.
2. Place the gel piece in a siliconized 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.
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 vacuum centrifuge for 15–30 minutes.
6. Add 70 µL of 40 mM ammonium bicarbonate in 9% acetonitrile.
7. Add 0.5 µL (0.5 µg) of SOLu-Trypsin solution.
8. Confirm 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.
11. If desired, an additional peptide extraction step may be performed on the gel piece. Add 50 µL of 0.1% trifluoroacetic acid in 50% acetonitrile and incubate for 30 minutes at 37 °C.
    Note: This extraction step only increases the peptide yield by about 5%.[6]
12. Remove the extraction solution and combine with the liquid from step 10.

References

1. Walsh, K.A., Meth. Enzymol., **19**, 41, (1970).
2. Smith, B.J., Methods in Molecular Biology, Volume 3, New Protein Techniques, Humana Press, (New Jersey: 1988) p 57.
3. Aitken, A. et al., Protein Sequencing: A Practical Approach, IRL Press, (Oxford, 1989) p 43.
4. Burdon, R.H., and Knippenberg, P.H., (eds.), Laboratory Techniques in Biochemistry and Molecular Biology: Sequencing of Proteins and Peptides, Volume 9, Elsevier, (New York, NY: 1989) p 73.
5. Stone, K.L. et al., A Practical Guide to Protein and Peptide Purification for Microsequencing, Academic Press, Inc. (New York, 1989) p 31.
6. Speicher, K. et al., J. Biomolecular Techniques, **11**, 74-86, (2000).

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