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ProductInformation

Trypsin Profile IGD Kit
For In-Gel Digests

Product Code **PP0100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

In-gel tryptic digestion of unknown proteins isolated by one dimensional or two dimensional polyacrylamide gel electrophoresis is a common tool used in proteomics. To determine or confirm their identities, proteins of interest are excised from the gel, digested with trypsin, and then analyzed by MALDI-MS or HPLC-MS with subsequent database searching. Trypsin is a pancreatic serine protease, 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] Tryptic digestion of the protein of interest results in a highly specific cleavage and a limited number of peptide fragments. The procedure detailed here is a modification of published protocols.[6,7]

The Trypsin Profile IGD Kit contains Proteomics Grade Trypsin that has been chemically modified through reductive methylation of the ε-amino groups of lysine to reduce autolysis and minimize autolytic fragments. In addition, it has been TPCK treated to remove residual chymotrypsin activity and then further purified by affinity chomatography, yielding a highly purified trypsin suitable for proteomics work.

This procedure has been optimized for polyacrylamide gels stained with Coomassie® Brilliant Blue, SYPRO™ Orange, or SYPRO Ruby dyes. For silver stained gels, a gel destaining step different than that used for dye stained gels is required. The ProteoSilver™ Plus Silver Staining Kit (Product Code PROT-SIL2) 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.

The Trypsin Profile IGD Kit provides adequate material to digest up to 100 samples.

Reagents

The Trypsin Profile IGD Kit is composed of 6 reagents:

- Destaining Solution – Solution to remove dye bound to the protein of interest. One bottle of powder that reconstitutes to a final volume of 75 ml (Product code D 9940).
- Trypsin Reaction Buffer – Buffer provides optimal pH for the trypsin digestion reaction. One bottle of powder that reconstitutes to a final volume of 11 ml (Product Code R 3527).
- Biotech grade acetonitrile – Solvent for preparation of other reagents. One 50 ml bottle (Product Code 49,444-5).
- Trypsin Solubilization Reagent – Reagent for reconstituting and stabilizing the enzyme. One vial containing 1 ml of reagent (Product Code T 2073).
- Peptide Extraction Solution – Solution for extracting peptides from the gel piece. One bottle containing 10 ml of solution (Product Code P 0743).
- Proteomics Grade Trypsin - The enzyme is supplied in five vials of 20 µg each (Product Code T 6567).

Equipment and Reagents Required But Not Provided

- ultra-pure water (18 megaohm or equivalent)
- flat nosed tweezers
- siliconized Eppendorf® tubes (Product Code T 4691 or equivalent)
- 37 °C heating block or heating bath
- scalpel (S 2771 and S 3021) or razor blade
- bench-top centrifuge (microcentrifuge)
- centrifugal concentrator (SpeedVac®)
- sonic bath
- ZipTip® pipette tips

Precautions and Disclaimer
This product is for laboratory use only, not for drug, household, or other uses. Consult the MSDS for information regarding hazards and safe handling practices. It is recommended to read the entire technical bulletin prior to starting the procedure.

Preparation Instructions
It is recommended to use ultrapure water (18 megaohm or equivalent) when reconstituting the reagents.

- Destaining Solution - Add 45 ml of water and 30 ml of Biotech grade acetonitrile to the bottle. After reconstitution, the bottle contains a solution of 200 mM ammonium bicarbonate and 40% acetonitrile.
- Trypsin Reaction Buffer – Add 10 ml of water and 1 ml of Biotech grade acetonitrile to the bottle. After reconstitution, the bottle contains a solution of 40 mM ammonium bicarbonate and 9% acetonitrile. Note: The Destaining Solution and the Trypsin Reaction Buffer do not need pH adjustment (pH is approximately 8.2).
- Trypsin Solubilization Reagent – Reagent contains 1 mM HCl and is ready to use.
- Peptide Extraction Solution – Solution contains 0.1% trifluoroacetic acid (TFA) in 50% acetonitrile and is ready to use.
- Trypsin Solution – Add 100 µl of the Trypsin Solubilization Reagent to one vial of trypsin. Mix the vial briefly to ensure the trypsin is dissolved. Add 900 µl of the Trypsin Reaction Buffer to the vial and mix. The final concentration of trypsin is 20 µg/ml. Note: Alternately, one vial of trypsin may be reconstituted with 100 µl of the Trypsin Solubilization Reagent (1 mM HCl) and stored at 2–8 °C for 2 weeks or at –20 °C for up to 4 weeks. When ready to prepare the working Trypsin Solution, an aliquot of the acidic trypsin solution may be combined with the correct amount of Trypsin Reaction Buffer
  (1 part of acidic trypsin solution to 9 parts of Trypsin Reaction Buffer).

Storage/Stability
This kit is stable for at least 1 year when stored at 2–8 °C. The Destaining Solution and the Trypsin Reaction Buffer are stable for up to one month after reconstitution when stored at 2–8 °C. The ammonium bicarbonate Trypsin Solution may be stored either at 2–8 °C for 2 weeks or as frozen aliquots for up to 4 weeks. The reconstituted Trypsin Solution is stable for at least 3 freeze-thaw cycles.

Procedure
The following procedure does not contain reduction or alkylation steps of the protein sample. For most in-gel digestion procedures, reduction and alkylation of the protein sample are suggested prior to running on the gel.[8] This sample treatment results in a 2D gel with less streaking and increased resolution, producing a gel with fewer artifacts and better reproducibility.

The ProteoPrep™ Reduction and Alkylation Kit (Product Code PROT-RA) contains reagents and procedures for reduction and alkylation of the protein sample during solubilization, equilibration for 2D electrophoresis, or in-gel tryptic digestion. Reduction and alkylation of the protein in solution prior to electrophoresis is most efficient.

The following procedure starts with a Coomassie® Brilliant Blue, SYPRO™ Orange, or SYPRO Ruby dye stained 1D or 2D polyacrylamide gel of a reduced and alkylated protein sample.

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 Peptide Extraction 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.
3. Cover the gel piece with 200 µl of Destaining Solution) 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 approximately 15 to 30 minutes.
6. Add 20 µl (0.4 µg of trypsin) of the prepared Trypsin Solution to the gel sample.
7. Add 50 µl of the Trypsin Reaction Buffer 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.

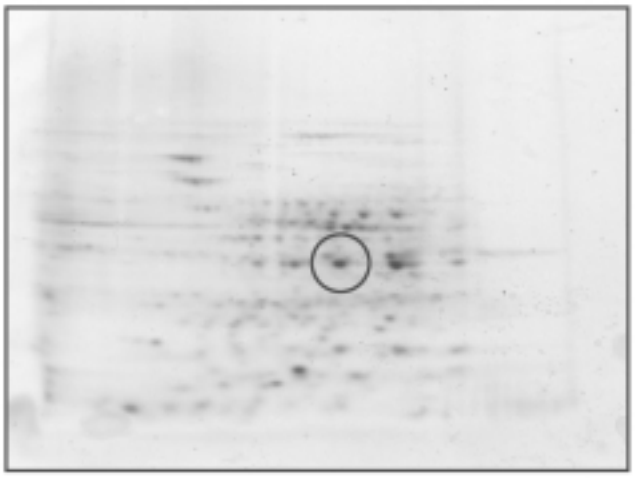
This solution contains the extracted tryptic peptides. If MALDI analysis is to be performed at this step, acidification with TFA prior to matrix addition may be needed.

11. Add 50 µl of the Peptide Extraction 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%.[6] 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.
12. Remove Peptide Extraction 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.

Results

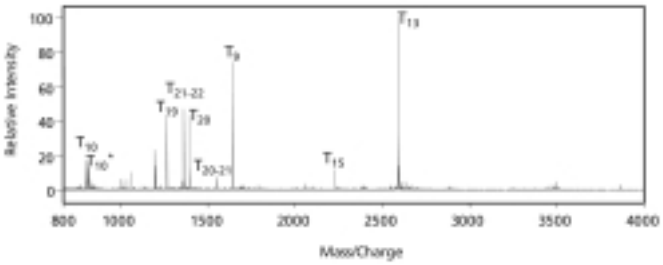
A solution of 2.5 mg/ml of *E. coli* cells (EC-1) in Cellular and Organelle Membrane Solubilization Reagent (Product Code C0356) was sonicated, then reduced with tributylphosphine and alkylated with iodoacetamide. A 109 µg protein sample was loaded on a 7 cm, pH 4-7, IPG strip (Product Code I 2906), focused for 50,000 Volt hours, then run on a 4-20% Tris-glycine SDS-PAGE gel at 150 Volts for 70 minutes. The gel was stained with EZBlue™ Gel Staining Reagent (Product Code G 1041) (See Figure 1).

Figure 1.
The 2D electrophoresis gel of a reduced and alkylated *E. coli* extract.



The indicated spot was cut out and digested with trypsin using the procedure for this kit. The peptide sample was desalted using a ZipTip™ C_{18} pipette tip and eluted directly onto the MALDI target using the MALDI matrix of 10 mg/ml of α-cyano-4-hydroxycinnamic acid in 70% acetonitrile with 0.03% trifluoroacetic acid. The MALDI analysis was performed in the reflectron positive ion mode (see Figure 2).

Figure 2.
The MALDI-MS of the Trypsin In-Gel Digested Spot from Figure 1.



The resulting monoisotopic masses were searched against the NCBI database at a tolerance of 150 ppm. The circled protein was identified as Outer Membrane Protein 3a from *E. coli* with the matched peptides providing 30% sequence coverage.

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 $lysine_{69}$ is not observed in the Proteomics Grade Trypsin, as it is fully converted to the dimethylated 2239.14 m/z peptide.

Related Products	Product Code
ProteoPrep Kits	
Total Extraction Sample	PROT-TOT
Membrane Protein Extraction	PROT-MEM
Universal Extraction	PROT-TWO
ProteoPrep Reduction and Alkylation Kit	PROT-RA
ProteoSilver™ Plus Silver Staining Kit	PROT-SIL2
ProteoMass™ MALDI-MS Calibration Kits	
Protein and Peptide	MS-CAL1
Peptide	MS-CAL2
Protein	MS-CAL3
EZBlue Gel Staining Reagent	G 1041
Bradford Reagent	B 6916
Bicinchoninic Acid Kit for Protein Determination	BCA-1
QuantiPro™ BCA Assay Kit	QP-BCA
Protein Standard Solution (1.0 mg/ml BSA)	P 0914

References

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