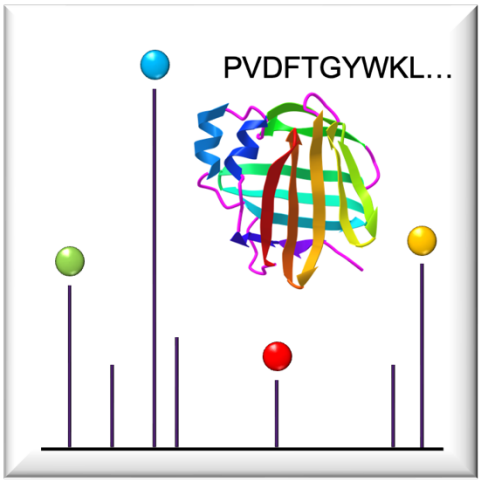
In-Gel Protein Digestion Protocol



Goal: Protocol to prepare SDS-PAGE protein gel bands for endopeptidase digestion and analysis by LC-MS/MS or MALDI-TOF for peptide sequencing.

Method:

1. Run protein sample on native or SDS-PAGE gels. Stain the gel with Coomassie Blue (G-250); for a comparison of Coomassie Blue formulations and their approximate detection limits (5-10 ng) see this link. Cut the Coomassie-stained gel band that contains the protein of interest into ~1 by 5 mm strips (lane width). Ensure to cut only the blue band.

2. Add 1 mL of destain solution: a 1:1 mixture of acetonitrile (MeCN) and 200 mM ammonium bicarbonate (ABC) buffer. Repeat the destaining process as necessary to completely remove the dye from the gel band. The destaining process can be carried out slowly at 4°C or quickly at 37°C

3. Add 1 mL of 50 mM ABC to 15 mg of DTT (reducing agent.) The DTT is provided in pre-weighed tubes that are stored in the -20 °C freezer.

4. 100 µL of the DTT solution to each gel band. Make sure that the solution covers the entire band. Heat the tube at 80°C for 10 minutes to reduce disulfide bonds and alkylate the cysteine residues.

5. Add 1 mL of 50 mM ABC to 18 mg of iodoacetamide (IAA, alkylating agent). The IAA is provided in pre-weighed amber tubes that are stored in the -20 °C freezer. Discard the DTT solution and replace it with 100 µL of the IAA solution. Make sure that the solution covers the entire band.

6. Store the gel tube in the dark for 1 hour to allow for alkylation.

7. Discard the IAA solution and replace it with 500 µL of 50 mM ABC. Let the tube sit for 15 minutes.

8. Discard the ABC solution and replace it with ~500 µL of destain solution. Let the tube sit for 15 minutes.

9. Discard the destain solution and replace it with 100 % MeCN. Let the tube sit for 5 minutes or until the gel band has shrunk and is white. Steps 7-9 help desalt the gel band prior to digestion.

10. Discard the ACN and let the gel tube air dry or briefly dry it in a Speedvac for 3 minutes.

11. Remove the frozen enzyme solution from the freezer. Trypsin is provided in 50 µL aliquots in 5 mM acetic acid at a concentration of 50ng/uL

12. Add 50 µL of 50 mM ABC to the trypsin to activate it for digestion.

13. Add 10-20 µL of trypsin gold to the dried gel tube. The gel should rehydrate without any remaining liquid in the tube.

14. Digest the gel at 37°C for 18 hours.

15. Extract the peptides from the gel band by adding 20 µL of 0.1% trifluoroacetic acid (TFA) for 15 minutes. Spot 1 µL of the extracted sample onto a MALDI target plate with either 2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinnamic acid (CHCA).

16. If the MALDI peaks look too weak or dilute, try adding 50 µL of ACN to the gel band extract. Wait for 15 minutes, remove the entire solution, and dry it in a Speedvac. Redissolve the sample in 10 µL of 0.1% TFA.

17. If the sample is too salty, use a C18 Zip-tip to remove the salt and concentrate the peptides for MALDI.

18. Respot the sample onto the MALDI target plate with DHB or CHCA.