In-gel Tryptic Digest for Protein ID by Mass Spectrometry

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This protocol is based on Shevchenko A, Wilm M, Vorm O, & Mann M. Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. Anal Chem 1996, 68:850-8. I have used it with success on both Coomassie and silver-stained gel bands. The procedure includes reduction and acetamidation steps that may be skipped if desired. For heavily stained Coomassie bands, it is helpful to wash gel pieces for 1 hr in 100 mM NH_4HCO_3 prior to dehydrating with acetonitrile (step 2).

1. Excise band from Coomassie or silver stained gel. Cut gel band into 1 mm cubes using clean razor blade on a clean glass surface. Transfer to an Eppendorf tube.

2. Remove excess water with pipet. Add 25-35 µL acetonitrile to tube to cover gel pieces. Incubate 10 minutes at RT to dehydrate and shrink gel pieces.

3. Remove acetonitrile with pipet. Speed-vac to dryness for 10 minutes.

4. Swell gel particles in 150 µL 10 mM DTT in 100 mM NH_4HCO_3. Incubate 1 hr at 56°C.

5. Cool to RT. Replace DTT solution with 150 µL 55 mM iodoacetamide in 100 mM NH_4HCO_3. Incubate 45 minutes at RT in the dark with occasional vortexing.

6. Remove solution and wash gel pieces with 150 µL 100 mM NH_4HCO_3. Incubate 10 minutes at RT.

7. Remove NH_4HCO_3 solution with pipet. Add 150 µL acetonitrile to dehydrate gel pieces. Incubate 10 minutes at RT.

8. Repeat wash steps 6 through 7. Remove acetonitrile and speed-vac to dryness for 10 minutes.

9. Place tubes in ice water bath and swell gel particles in 25-35 µL digestion buffer. Incubate 45 minutes in ice water bath. Digestion buffer consists of 12.5 ng/µL trypsin (Promega sequence-grade

modified porcine trypsin, Cat. #V511A) in 50 mM NH4HCO3. To make the digestion buffer, dissolve 20 µg Promega trypsin in 80 µL Promega trypsin buffer solution (50 mM acetic acid), and dilute with 50 mM NH4HCO3 to 12.5 ng/µL.

10. Remove trypsin-containing buffer. Add 5-10 µL 50 mM NH4HCO3 without trypsin to keep pieces wet during cleavage. Incubate o/n at 37°C.

11. Spin 1' at 14,000 rpm to spin down gel pieces. Save supernatent in a separate PCR tube.

12. Add 20 µL 20 mM NH4HCO3 to cover gel pieces. Incubate 10 minutes at RT. Transfer supernatent to the PCR tube from step 11.

13. Add 25 µL 5% formic acid, 50% acetonitrile to the gel pieces. Incubate 20 minutes at RT.

14. Spin 1' at 14,000 rpm. Remove formic acid/acenonitrile solution and save in the same PCR tube from step 11.

15. Repeat formic acid extraction (steps 13 through 14) twice more.

16. Dry PCR tube in speed-vac to complete dryness. Store at -20°C until analysis.