In-gel Digestion of Coomassie-Stained Proteins from 1D or 2D Gels (No Reduction & Alkylation)

Note: The following protocol should be considered as first starting point. For each research projects experimental conditions should be critically evaluated and, if necessary, changes should be made to improve performance and efficiency.

Tips:

In order to minimize keratin contamination wear gloves and avoid touching the gels, don't allow hairs to fall on gels, don't wear woolen sweaters etc., don't speak, laugh or spit on gels during gel handling and excision.

If possible use commercially available ready-to go products, use freshly prepared reagents and work in a laminar flow hood.

Step-by-step Protocol:

1. Excise gel bands or spots.

2. It is highly recommended that a positive and negative control be conducted in parallel to all analyses. The positive control can be a marker or known protein while the negative control should be a blank region of the gel.

3. Wash 2x with 50µL of 18 MegaOhm water for approx 15 minutes.

4. Wash sufficient times with 50 µL of a 50% acetonitrile/25mM ammonium bicarbonate to remove all stain from gel plug. Each wash should be approximately 15-30 minutes in length. Two washes should be sufficient for moderately stained bands/spots.

5. Wash and dehydrate with 50 µl (spot) to 100 µL (band) of acetonitrile until gel plugs turn opaque, needs approximately 5-15 minutes.

6. Decant all liquid from opaque gel plugs

7. Dry sample plugs in vacuum centrifuge.

8. Prepare trypsin stock solution while drying samples using sequence grade, modified porcine Trypsin (Promega). Prepare trypsin stock solution by dissolving 20 to 25 µg in 200µL 1mM HCl or 1% Acetic acid so that final concentration is between 100ng/mL to 125 ng/µL.

9. Prepare working trypsin solution by diluting stock solution 1 to 10 with 25 mM ammonium bicarbonate such that final concentration = 10 to 12.5 ng/µL; use directly.

Note: Note the pH difference in stock solution (~2.9) versus working solution (pH~8.0). Trypsin activity is pH dependent. Trypsin working solutions must be used directly after increasing pH to 8.

10. Rehydrate dried gel plug/bands in approximately 20 µL trypsin working solution for 45 minutes on ice. This allows the trypsin to infuse into the gel plug for "in-gel" digestion.

11. Remove excess trypsin solution with pipette.

12. Add 10 to 20 µL of 25mM ammonium bicarbonate to sample vial to ensure proper hydration during digestion at 37^0C.

Digest at 37°C for 4 to 6 hrs or overnight.

Tip: Overnight digestions are possible but often yield higher levels of autolytic trypsin peptides which may interfere during automated tandem mass spectrometry analysis if very low level protein IDs should be obtained.

13. Recover supernatant.

Note: One can use this solution directly for mass spec analysis; But we observed that additional extraction yields frequently more peptides.

14. Extract twice with of 50% acetonitrile/25mM ammonium bicarbonate for 15 minutes to recover additional peptides. Pool with supernatant from step 13..

15. Extract with 25 µL to 50 µL of 100% acetonitrile. Pool with supernatant from step

16. Concentrate to 5 µL or dryness in vacuum centrifuge.

Note: Concentration to dryness is more convenient but may result in some loss of peptides which will not resolubilize.

17. Dried peptides can be stored at -20°C or -80°C.

Mass Spec Analysis:

18. Immediately prior to mass spectrometric analysis dissolve dried peptides in 50% acetonitrile/1% formic acid.

Note: Alternatively, 0.1% trifluoroacetic acid can be used but TFA can reduce ionization efficiency through ion pair formation.