"In-Gel" Trypsin Digestion Protocol for Proteins in SDS-PAGE Gel Slices

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Samples to be digested in the gel are run in as few lanes as possible to maximize the concentration of the protein within the bands of interest. The gel is stained in 0.1% Coomassie R250/20% MeOH/0.5% AcCOOH, and then destained in 30% MeOH until the bands are visible and the background is nearly clear. Volumes and reagent quantities described herein are for one band from a 1 mm gel slice. A reduction and alkylation step is included. Alternatively, the sample may be reduced and alkylated prior to electrophoresis. Note that an alternate buffer system is also provided for LysC digestion.

1. Wash the gel slices for at least 1 hr in 500 µl of 100 mM ammonium bicarbonate. Discard wash.

2. Add 150 µl of 100 mM ammonium bicarbonate and 10 µl of 45 mM DTT. Incubate at 60°C for 30 min.

3. Cool to room temp and add 10 µl of 100 mM iodoacetamide and incubate for 30 min in the dark at room temperature.

4. Discard the solvent and wash the gel slice in 500 µl of 50% acetonitrile/100 mM ammonium bicarbonate with shaking for 1 hr. Discard the wash. Cut the gel into 2-3 pieces and transfer to a 200 µl eppendorf style PCR tube.

5. Add 50 µl of acetonitrile to shrink the gel pieces. After 10-15 min remove the solvent and dry the gel slices in a rotatory evaporator.

6. Re-swell the gel pieces with 10 µl of 25 mM ammonium bicarbonate containing Promega modified trypsin (sequencing grade) at a concentration such that a substrate to enzyme ratio of 10:1 has been achieved. (If the amount of protein is not known, add 0.1-0.2 micrograms of modified trypsin in 10 µl of 25 mM ammonium bicarbonate). After 10-15 minutes add 10-20 µl of additional buffer to cover the gel pieces. Gel pieces need to stay wet during the digest. Incubate 4 hrs to overnight at 37°C.

Proceed to step 8 if further extraction of the gel is desired (recommended) - otherwise continue with step 7.

7. Approximately 0.5 µl of the supernatant may be removed for MALDI analysis and/or the supernatant acidified by adding 10% TFA to a final concentration of 1% TFA for injection onto a narrowbore or microbore reverse phase column. (If necessary the sample’s volume may be reduced ~1/3 on a rotatory evaporator.)

8. Extraction (Optional): Save supernatant from step 7 in tube X, and extract peptides from gel twice with 50 µl of 60% acetonitrile/0.1% TFA for 20 min. Combine all extracts in tube X (using the same pipet tip to minimize losses), and speed vac to near dryness. Reconstitute in 20 µl of appropriate solvent. Proceed with chromatography or MALDI analysis.

Alternate Digestion Protocol for LysC Digestion

If a LysC digest is desired a different buffer system is used, using the same volumes as before. This buffer system (below) has been used successfully with LysC from Achromobacter lyticus. LysC from other sources has not been tested.

Wash the gel pieces in 500 mM Tris-HCl, pH 9.2/50% acetonitrile. Digest the protein(s) in the gel slice with LysC in 100 mM Tris-HCl, pH 9.2.