"In-Gel" Digestion of Proteins in SDS-Page Gel Slices for Mass Spec

Protocol revised by Lori, June 2011

We recommend Gelcode Blue® Coomassie stain (Pierce) for detecting bands. This technique works for any band that can be seen by this stain. 0.1 to 0.2 micrograms of protein is ideal. Use a new scalpel or razor blade to cut out each band. Mince each band into < $1mm^2$ pieces and transfer to a clean microcentrifuge tube.

CAUTION: Many silver stains, including those claiming mass spec compatibility, give poor results. Collodial Coomassie stains such as that recommended above work well. If you feel compelled to stain with silver, the band should be completely destained (no visible color) before starting this protocol.

Note: To avoid contaminants use only MilliQ water (or better) and wear gloves throughout preparation. All reagents should be HPLC grade. Prepare all solutions fresh.

1. Wash the gel pieces for 20 min. in 500µl of 100mM NH_4HCO_3. Discard the wash.
2. Add 150µl of 100mM NH_4HCO_3 and 10µl of 45mM DTT. Incubate at 50°C for 15 min.
3. Cool to room temperature and add 10µl of 100mM iodoacetamide and incubate for 15 min. in the dark at room temp.
4. Discard the solvent and wash the gel slice in 500µl of a 50:50 mix of acetonitrile and 100mM NH_4HCO_3 with shaking for 20 min. Discard the solvent.
5. Add 50µl of acetonitrile to shrink the gel pieces. After 10-15 min., remove the solvent and dry the gel fragments in a speed vac.
6. Reswell the gel pieces with 10µl of 25mM NH_4HCO_3 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 10-20µl of 25mM NH_4HCO_3 containing 0.1-0.2µg of trypsin) After 10-15 min., add 10-20µl of additional buffer, enough to cover the gel pieces. Incubate overnight (8 hours or more) at 37°C.
7. Remove the supernatant and place in new microcentrifuge tubes. Extract remaining peptides from the gel pieces twice with 50µl of 60% acetonitrile/0.1% formic acid for 20 min, then once with 25µl acetonitrile. Add these extracts to appropriate tubes containing the supernatant of the sample. Speed vac to dryness.