

“IN-GEL” DIGESTION OF PROTEINS IN SDS-PAGE GEL SLICES FOR MASS SPEC

Revised June 2006 by Lori.

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 < 1mm² pieces and transfer to a clean microcentrifuge tube.

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 NH₄HCO₃. Discard the wash.
2. Add 150μl of 100mM NH₄HCO₃ 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 NH₄HCO₃ 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 NH₄HCO₃ 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 0.1-0.2μg of trypsin in 10μl of 25mM NH₄HCO₃) After 10-15 min., add 10-20μl of additional buffer 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. Add these extracts to appropriate tubes containing the supernatant of the sample. Speed vac to dryness.

Solutions:

100mM NH₄HCO₃

for 100ml:
0.79g
ddH₂O to 100ml
prepare fresh