Protocol for Preparation of Coomassie G-250-stained 2D Gel Spots for In-gel Trypsin Digestion

I. Washing/Destaining

a. The SDS-PAGE gel should only be stained with high quality Coomassie Brilliant-Blue G-250. Use only MilliQ water for destaining and reagent preparation.

b. Once gel is destained and background is minimal, excise stained spots and transfer into a clean 1.5 mL Eppendorf microcentrifuge tube.

c. Wash gel with 500 µL of wash solution (50% HPLC grade acetonitrile, 50mM ammonium bicarbonate) and incubate at room temperature for 15 min with gentle agitation (vortex mixer or shaker on lowest setting). Remove solution with a pipette and discard.

d. Wash gel two more times with 500 µL of wash solution (15 min each) or until the Coomassie dye has been completely removed (usually 4 washes with G-250 coomassie).

e. Dehydrate gel in 500 µL 100% HPLC grade acetonitrile briefly. Remove with pipette and discard, taking care not to pull gel piece(s) into pipette tip.

f. Completely air-dry each gel piece with the cap just slightly open. Care should be observed when handling the tube once the gel is dry because it will “jump” out due to static electricity.

II. Digestion/Extraction

a. While gel is drying prepare protease digestion solution. [Typically, this is modified sequencing grade trypsin (Product number V5111, Promega, Madison, WI)]. Resuspend lyophilized trypsin (20 µg/vial) in 20 µL of the 50mM acetic acid solution provided with trypsin, yielding a 1 µg/µL stock solution. Dilute that stock to 1 µg/50 µL with 50mM ammonium bicarbonate (50 fold dilution), keeping in mind the number of gel pieces you have to digest. Store the remaining trypsin stock at –70° C. Do not freeze-thaw trypsin stock solutions more than once.

b. Rehydrate the gel with a minimal volume of trypsin protease digestion solution. Use 20 µL for small gel pieces, 30-40 µL for larger gel pieces. Add more if gel pieces absorb all the liquid.

c. Incubate at 4°C (on ice) for 1 hour. At this point most of the trypsin will be absorbed into the gel pieces.

d. Remove trypsin solution and replace with just enough 50mM ammonium bicarbonate to cover gel pieces. Incubate at 37°C for 16-18 hours (overnight).

e. Add 7 μL of 1% formic acid to stop the action of trypsin. Spin down the sample and transfer supernatant (containing tryptic peptides) to a clean 0.5 mL Eppendorf microcentrifuge tube and keep supernatant on ice.

f. Rinse the gel pieces with a small volume of 0.1% formic acid and transfer solution to tube from step IIIe.

g. Store the samples at -20°C until ready for analysis.

Notes

- This protocol does not contain a reduction and alkylation step, and assumes these steps have been performed during IPG strip equilibration, prior to running the second dimension.
- Always use non-latex gloves when handling samples, keratin and latex proteins are potential sources of contamination.
- Never re-use any solutions, abundant proteins will partially leach out and contaminate subsequent samples.

References

1. Havlis J, Thomas H, Sebela M, Shevchenko A: Fast-response proteomics by accelerated in-gel digestion of proteins. *Analytical Chemistry* **75**: 1300-1306 (2003).

2. Jiménez CR, Huang L, Qiu Y, Burlingame AL: In-gel digestion of proteins for MALDI-MS fingerprint mapping. In: Coligan JE (ed) Current Protocols in Protein Science, pp. 16.4.1-16.4.5. John Wiley & Sons, Inc., Brooklyn, N.Y. (1998).