Protocol for Preparation of Coomassie G-250-stained 1D Gel Bands 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. Wash 1.5 mL Eppendorf microcentrifuge tubes with 50% HPLC grade acetonitrile:MilliQ water.

c. Excise gel band(s), cut into 1mm pieces and place into a washed tube(s).

d. Equilibrate gel pieces with 500 µL 100 mM ammonium bicarbonate (aq) buffer for 20 minutes at room temperature with gentle agitation.

e. Wash gel pieces with 500 µL wash solution (50 mM ammonium bicarbonate, 50% acetonitrile) until Coomassie is removed (3 - 4 x 15 min, with gentle agitation).

f. Rinse gel pieces briefly with 500 µL 100% HPLC grade acetonitrile. Discard acetonitrile, and allow gel pieces to air-dry.

II. Reduction/Alkylation

a. Reduce the in-gel protein with dithiothreitol (150 µL 10mM DTT/ 100mM ammonium bicarbonate) for 30 minutes at 56° C.

b. Cool the sample to room temperature, remove and discard DTT solution.

c. Alkylate the in-gel protein with iodoacetamide (100 µL 50 mM IAA/100 mM ammonium bicarbonate) in the dark at room temperature for 30 minutes. Remove and discard IAA solution.

d. Wash sample at room temperature for 15 minutes with 500 µL of wash solution.

e. Rinse gel pieces briefly with 500 µL 100% HPLC grade acetonitrile. Discard acetonitrile and allow to air-dry.

III. Digestion/Extraction

a. Rehydrate gel pieces for 60 minutes at 4°C (on ice) in 30 µL of 0.02 µg/µL trypsin (Promega, modified porcine, TPCK-treated, Cat#: V5111) in 50mM

ammonium bicarbonate. (Be sure that enough volume is added to ensure complete rehydration of gel pieces)

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

c. 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.

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

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

Notes

- 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

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