Protocol for Preparation of Silver-Stained 1D Protein Bands for In-Gel Tryptic Digestion

I. Washing/Destaining

a. Wash 0.5 mL Eppendorf microcentrifuge tubes with 50% HPLC grade acetonitrile, 0.1% trifluoroacetic acid (TFA).

b. Using a freshly stained gel, cut the protein bands at the margin of the visible bands using a new razor blade. Wash the razor blade after cutting each band. Cut gel band into 1mm cubes and place gel pieces in pre-washed 0.5 mL microcentrifuge tube.

c. Add 200 μL MilliQ water and shake for 1 hour to remove the acetic acid. Remove and discard supernatant.

d. Destain by adding 25 μl of 15mM potassium ferricyanide and 25 μL of 50mM sodium thiosulfate. Shake gently until the band pieces go clear (**i.e. until all the silver is removed**). Remove and discard the supernatant. The gel pieces will now appear yellow in color.

e. Add 200 μL of 200mM ammonium bicarbonate, pH 8. Shake for 15 minutes. Gel pieces should be colorless. Remove and discard supernatant.

f. Add 200 μL of 50:50 (v/v) 200 mM ammonium bicarbonate: acetonitrile. Shake gently for 15 minutes. Remove and discard supernatant.

g. Dehydrate gel piece by adding 200 μL HPLC grade acetonitrile. Remove immediately and discard supernatant. Allow residual acetonitrile to evaporate completely.

II. Reduction/Alkylation

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

b. Allow sample to cool to room temperature, remove and discard DTT solution.

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

d. Add 200 μL of 50:50 (v/v) 200 mM ammonium bicarbonate: acetonitrile. Shake gently for 15 minutes. Remove and discard supernatant.

e. Dehydrate gel piece by adding 200 μL HPLC grade acetonitrile. Remove immediately and discard supernatant. Allow residual acetonitrile to evaporate completely.

III. Digestion/Extraction

a. Rehydrate each gel piece at 4°C in 10-20 μL 0.02 μg/μL of Promega Sequencing Grade Modified Trypsin prepared in 50mM ammonium bicarbonate. If additional solvent is required to cover gel piece, use 50 mM ammonium bicarbonate (NO Trypsin).

b. Close the microcentrifuge tubes tightly and incubate overnight at 37°C.

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

- Digestion protocol may be conveniently stopped when the water is added before shaking (step Ic). Freeze the bands/spots solution at -20°C. When ready to continue digestion, thaw bands/spots and shake for 1 hour.
- 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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