In-gel digests for MALDI-TOF analysis using Coomassie Blue and Silver stained gel slices

Destain Silver Stained Gel slices

(Gharahdaghi, F., Weinberg, C.R., Meagher, D.A., Imai, B.S. and Mische, S.M. (1999) Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: a method for the removal of silver ions to enhance sensitivity. Electrophoresis 20: 601-605.)

Procedure

1.) Soak stained gel in 0.5-1 ml volume of a 1:1 mixture of 30mM potassium ferricyanide: 100mM sodium thiosulfate for 5 minutes (until silver stain disappears).

2.) Rinse gel slices with 3 x 1 ml of water until the yellow color disappears.

In-gel digestion

(Shevchenko, A., Tomas, H., Havlis, J., Olsen, J.V. and Mann, M. (2006). In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nature Protocols 1: 2856-2860.)

Procedure

Reduction and Alkylation (performed in coated 500 ul Eppendorf tube).
(This is useful for proteins with a lot of Cys that may not become alkylated by free acrylamide)

1.) Soak gel slice in water to remove residual acid for about 30 minutes. (Optional if gel is in water).

2.) Cut gel into 1x1 mm pieces. (Optional if gel plug is small).

3.) Add 10 ul of 250mM Ammonium Bicarbonate. (Adjust volume as need to just cover bits).

4.) Add 10 ul of 45mM DTT and react for 30 minutes and 50o C.

5.) Cool to room temperature and add 10 ul of 100mM Iodoacetemide (IDA) and react for 30 minutes **IN THE DARK**.

6.) Remove residual supernatant and proceed with gel Dehydration.

Dehydration of gel slices

1.) Equilibrate the stained gel slice in 0.5 ml of water for 30 minutes to remove any residual acid.

2.) Cut the gel slice into 1x1 mm pieces (not necessary for small 2D spots).

3.) Place pieces in 0.5 ml of 50% acetonitrile: 50% 50mM ammonium bicarbonate; mix occasionally. (Prepare from 100mM stock of ammonium bicarbonate). Soak for a minimum of 30 minutes.

4.) Replace supernatant with 200 ul of acetonitrile to completely dehydrate for 30 minutes; mix occasionally. (Gel slices should turn opaque white).

5.) Remove acetonitrile and dry gel chips in the Speed Vac, 5 minutes, no heat.

Tryptic Digest

1.) Rehydrate slices in 20 ul modified Trypsin (prepared 20 ugm/ ml in 25mM ammonium bicarbonate, 0.001% n-octylglucoside*). Add excess 25 mM ammonium bicarbonate to cover slices completely, if necessary. Leave in an ice bucket or fridge.

2.) After approximately 30 minutes, check if all solution was absorbed and add more trypsin buffer if necessary. Gel pieces should be completely covered with trypsin buffer.

3.) Leave gel pieces for another 90 minutes to saturate them with trypsin and then add 10-20 ul of ammonium bicarbonate buffer to cover gel pieces and keep them wet during enzymatic cleavage.

CRITICAL STEP: although after approximately 30 minutes dried gel pieces do not absorb any more buffer, the yield of tryptic peptides increases substantially while extending the incubation time, presumably because of slow diffusion of the enzyme into polyacrylamide matrix.

4.) Incubate overnight at 37C. Remove supernatant and save for analysis.

5.) Soak slices with 50 ul of 80% acetonitrile: 1% formic acid. Slices will shrink. Leave for 10 minutes.

6.) Remove supernatant and pool with supernatant in step 4.

7.) Dry down digest in Speed Vac. Stop when digest is *almost* dry (~ 10 ul).

8.) Add 1 ul of 1% TFA to insure acidification of digest.

9.) Desalt on a Millipore Zip Tip (u-C18) according to instructions. Elute from the Zip Tip with a small volume (~1-2 ul) of 0.1% TFA: 50% acetonitrile.

Sample is ready for MALDI-TOF analysis.

*Addition of 0.0001% n-octylglucoside has been known to improve recoveries of larger more hydrophobic peptides.

Adapted from: Shevchenko, et al, 2006, Nature Protocols, 1:6, 2856-2860.