In-gel trypsin digestion

Adapted from: Shevchenko A., Wilm M., Vorm O., Mann M. (1996) Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels. Anal.Chem.

1. Excision of bands from polyacrylamide gels:

Excise the band/spot of interest from the gel. Cut as close as possible to reduce the amount of “background” gel. Cut the excised piece into 1x1 mm cubes and transfer them to a 1.5 mL tube.

2. Washing of gel pieces

Wash the gel pieces with 300-800 µl of 70% 50mM Amm.Bic./30 % Acetonitile for 30-60 minutes. The volume of washing solution and the washing period depends on the degree of Coomassie staining and the size of the gel slices. For weakly stained Coomassie gel pieces washing with 300 µl for 30 minutes is sufficient. For "dark blue" Coomassie bands and large slices, gel-pieces should be washed for 60 minutes with 800 µl washing solution.

Remove solution.

Repeat this step if necessary until all Coomassie is removed.

Shrink the gel pieces with 300-800 µl Acetonitile for 10 minutes.

Wash the gel pieces for 10 minutes in 50mM Amm.Bic.

Shrink the gel pieces with 300-800 µl Acetonitile for 10 minutes.

Remove the Acetonitrile and dry the gel particles for 3-5 minutes in a vacuum centrifuge.

3. Reduction and alkylation

Swell the gel particles in 15-200 µl (depending on the volume of gel pieces, don't use more than necessary) 10 mM DTT/50mM Amm. Bic. and incubate for 45 minutes at 56 degrees to reduce the proteins.

Chill tubes to room temperature. Remove excess liquid (if there is a substantial excess) and replace it quickly with 15-200 µl (same volume as was used for the DTT) of freshly prepared 55 mM iodoacetamide in 50mM Amm. Bic.. Incubate for 30 minutes at room temperature in the dark and remove solution.

4. Washing of gel-pieces

Wash the gel pieces in 400-800 µl of 50mM Amm. Bic. for 15 minutes. Remove solution.

Shrink the gel pieces with 300-800 µl Acetonitile for 10 minutes.

Wash the gel pieces for 10 minutes in 50mM Amm.Bic.

Shrink the gel pieces with 300-800 µl Acetonitile for 10 minutes. Remove the Acetonitrile and dry the gel particles for 3-5 minutes in a vacuum centrifuge.

5. In-gel digestion

Rehydrate gel pieces in 15-200 µl (depending on the volume of gel pieces and the amount of proteins in the gel pieces) of 15 ng/µl of trypsin (Promega or Roche, sequencing grade) in 25 mM Amm. Bic./1mM $CaCl_2$. Incubate at 37 degrees for 10-16 hours.

6. Extraction of peptides

Shake the gel pieces for 1 hours in 100-800 µl (depending of the size of the gel slices) of 5% formic acid, 30 % AcN, then sonicate for 5 minutes. Transfer the solution to a fresh Eppendorf tube and wash the gel pieces with 100-500 µl of AcN in a shaker. Combine the AcN washing fraction with the previous fraction. It is important that no small gel pieces are transferred together with the extraction buffers. Dry the samples in a vacuum centrifuge and store at -20°C. Samples should be desalted afterwards.