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nanoLIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION-QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (nanoLC-ESI-qTOF-MS)

We identify proteins in polyacrylamide gels (PA), lyophilised samples or solutions. The proteins are reduced, alkylated and fragmented with trypsin. The peptide mixtures are desalted with homemade C18-StageTips and analysed on a **nanoHPLC (Agilent Technologies, Germany) coupled with Compact ESI-qTOF mass spectrometer (Bruker Daltonik GmbH , Germany;** https://www.bruker.com/en/products-and-solutions/mass-spectrometry/qtof/compact.html). Proteins are identified with MaxQuant and/or Mascot search engines using publicly available or user-supplied protein sequence databases.

Sample preparation

1. Prepare the samples in a dust-free environment to avoid excessive contamination with keratin.
2. Samples should contain at least 0.1 μg of protein. Protein spots/bands should be clearly visible on SDS-PAGE gels after staining with one of the MS-compatible staining methods (Coomassie Brilliant Blue, colloidal silver, SYPRO Ruby stain or imidazole-SDS-$Zn2^{+}$ reverse stain).
3. Peptide samples must be free from compounds that suppress ionisation (salts, buffers and surfactants). Use dialysis, StageTip/ZipTip or other procedure to remove impurities.
4. The following procedure for **silver staining** is recommended:
    1. Fix the proteins in a gel in 30% (v/v) ethanol and 10% (v/v) acetic acid for at least 30 minutes. In the case of 2-DE gels, overnight fixation is necessary to remove the carrier ampholytes.
    2. Wash the gel several times with Milli-Q water and leave it in the water for 10 minutes.
    3. Sensitise the gel in 0.5 mg DTT/100 mL Milli-Q water for 30 minutes.
    4. Stain the gel in 0.1 g silver nitrate/100 mL Milli-Q water for at least 60 minutes.
    5. Rinse the gel briefly with Milli-Q water and a developer (see below for composition).
    6. Develop the gel with 28.5 g sodium carbonate, 0.5 mL formaldehyde (37% (v/v))/L Milli-Q water until the protein spots are clearly visible.
    7. Stop staining the gel with 5 g solid citric acid per 100 mL of developer.
    8. Wash the gel in Milli-Q water, changing it several times.

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