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EDMAN SEQUENCING OF A PROTEIN/PEPTIDE SAMPLE

Sequence analysis of a protein/peptide sample is performed using a **PPSQ-53A Gradient System** (Shimadzu, Japan). PTH amino acid derivative of the N-terminal amino acid residue is prepared using Edman chemistry. Analysis of PTH-amino acid derivatives is performed on the Wakopak® Wakosil PTH-GR (S-PSQ; 2.0x250mm) (FUJIFILM Wako Pure Chemical Corporation, Japan) using the HPLC pump LC-20AD with the degassing unit DGU-20A3R. Reagents and solvents are of sequencing grade (FUJIFILM Wako Pure Chemical Corporation, Japan). Data are analyzed by LabSolutions PPSQ software (Shimadzu, Japan).

We accept protein/peptide samples in solution, lyophilised or adsorbed on the PVDF membrane.

Liquid and lyophilised samples

1. 10–50 pmols of a protein/peptide is required for reliable analysis, although a lower amount could be analysed (~1 pmol) if the sample is homogenous.
2. Solvent should be volatile (water, acetonitrile, propanol, acetic acid or formic acid) and free of: compounds containing free amino groups (*e.g.* Tris), salts in higher concentrations (sulphates or phosphates), detergents (SDS is acceptable in a low concentration, up to 0.03% (m/v)) and compounds that absorb in the UV region (Triton X-100 and other aromatic compounds).
3. Dialysis or HPLC analysis of a sample is recommended to remove the above mentioned contaminants. Samples could be shipped in lyophilized form or in 15–30 µL of solution.
4. For dried samples, solubility data should be provided.

Samples on the PVDF membrane

1. Use pre-cast polyacrylamide gels for (SDS or native) gel electrophoresis or cast the gel one day before use. This guarantees complete polymerization of the gel and reduces contents of oxidants and free radicals in the gel, which minimizes the possibility of the N-terminal protein/peptide blockage. In order to minimize formation of aldehydes, which also block Edman sequencing, use the HPLC-grade glycerol to prepare the sample buffer. The sequencing yield will be also higher if you just heat your sample at 37°C for 15 min and NOT boil it before you apply it on the gel. Apply at least 1 µg of protein to be sequenced.
2. Blot proteins onto the PVDF membrane (do NOT use nitrocellulose membranes).
3. Wash membrane thoroughly with deionized water to remove excessive glycine and stain the membrane with Coomassie Brilliant Blue (CBB), Ponceau S or Amido Black (do NOT stain with silver).
4. The following procedure is recommended for CBB staining:
    1. Put the PVDF membrane into a staining solution (0.1% (m/v) CBB R-250 / 0.5 - 1% (v/v) acetic acid / 40% (v/v) methanol) for maximum 1 min.
    2. Destain the membrane in 50% (v/v) methanol. Exchange the destaining solution several times.
    3. Wash the membrane with dH_2O and let it dry.
    4. Cut out clearly visible bands/spots.
    5. Store PVDF membrane samples in the Eppendorf Safe Lock tube at -20°C. PVDF samples can be shipped at room temperature.

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