Sample preparation

- Protein sequencing analysis by N-terminal Edman degradation requires minimum amount ~10-50 pico moles of a pure protein/peptide. Samples can be analyzed either attached to PVDF membranes or in solution.

  If a protein sample contains more than one protein, the analysis will show a mixture of amino acids in each Edman degradation cycle, and a sequence cannot be determinated.

- **Liquid samples**

  1. The chromatographic protein purity should be >90%.
  2. Sample should be salts and detergents free.
  3. The sample should be in a volatile solvent or buffer (0.1% TFA, acetic acid, trimethylamine, ammonium acetate, ammonium bicarbonate, H_2O, acetonitrile, propanol …)
  4. Avoid lyophilizing the sample. This can lead to substantial loss of sample for some proteins. It is more practical to concentrate the sample in a speed-vacuum.
  5. Put the protein/peptide into polyethylene or polypropylene micro centrifuge tubes. The sample volume should be < 200 µl.
  6. Freezing or refrigerating the liquid sample for cold shipment is at the discretion of the investigator.

- **Electro-blotted samples onto PVDF**

  1. Use pre-cast gels or let the gel polymerize overnight before running the sample. This will help eliminate any N-terminal blockage due to unpolymerized acrylamide in the gel. Be sure to use high-grade methanol as well.
  2. Always try to have as much protein as you can in as small an area of PVDF membrane as possible. The piece of PVDF should be no larger than 3 x 10 mm and the number of pieces no more than four. Keep in mind that transfer is not 100% efficient so load an adequate amount of protein or peptide onto the gel prior to electrophoresis. Remember that the intensity of the stain is not indicative of sample amount and that the presence of a single band does not always indicate that there is only one species present.
  3. After electro-blotting, the membrane should be stained with Coomassie Blue R-250*, Ponceau S$^{\blacklozenge}$ or Amido black$^{\#}$ (do not use silver staining). It is advisable not to over-stain and use fresh dye solutions.

4. Destain the membrane and rinse thoroughly with Milli-Q H_2O to lower the very high concentrations of Tris, glycine, and other gel and transfer buffers that otherwise will interfere with sequencing.

5. After drying at room temperature, the bands of interest should be excised with a clean razor blade, taking care not to cut off empty membrane without protein, and placed in 1.5 ml Eppendorf type micro tube for shipment.

**Coomassie blue R-250 (0.1% solution in 40% methanol/10% acetic acid) staining is carried out for 5 minutes followed by destaining (5-10 minutes) in a methanol solution (50% methanol).*

♦Ponceau S (0.2% solution in 1% acetic acid) staining is carried out for 1-2 minutes followed by a simple rinse with deionized water.

#Amido Black (0.1% Amido Black in 30% methanol/ 10% acetic acid). Destain by several washes in distilled water.

A rough and ready guide is if you can photocopy the bands then they can be sequenced.

- All reagents and solvents must be of the highest purity available (HPLC grade, sequencing grade and electrophoresis grade). Avoid molecular biology grade reagents.
- Always wear gloves and work in a clean dust-free area. Dust and unprotected hands are the major sources of amino acid contamination.

Guidance to choose the required number of degradation steps:

- ✓ 4-6 residues (degradation steps) to determine a cleavage site in a known protein
- ✓ 5-10 residues to confirm the identity of an isolated known protein
- ✓ 10-20 residues to design oligonucleotide probes
- ✓ 15 or more residues to identify an unknown protein

References

Kaye D. Speicher, Nicole Gorman, and David W. Speicher. N-Terminal Sequence Analysis of Proteins and Peptides. *Current Protocols in Protein Science* 11.10.1-11.10.31. Published online August 2009 in Wiley Online Library .DOI:10.1002/0471140864.ps1110s57 http://onlinelibrary.wiley.com/doi/10.1002/0471140864.ps1110s57/abstract

Paul T. Matsudaira. A Practical Guide to Protein and Peptide Purification. Academic Press, Inc. San Diego, California. 1993.