Edman Chemical Sequencing

Sample preparation for Edman sequencing from PVDF membrane.

The protocol guidelines below are not a substitute for a discussion of your project. Always call to discuss the specifics of your project before submitting a sample. Successful projects stem from a thorough understanding by both the researcher and our laboratory of goals, expectations and requirements.

- Edman sequencing requires 10pmol or more protein for high quality extended sequence runs. (See Protein Quantity Estimation). Maximize quantity of sample you can commit to this experiment.

- Once a certain quantity has been achieved, density is the second most important factor, as the reaction cartridge is only 9mm wide and can hold only a few 1x8mm strips of PVDF before the flow is impeded in the instrument. Maximize density of the final gel bands.

- Reduce background proteins with fresh preparations of all gel based buffers, stains and wash solutions. Segregated or new glassware and gloves should be used for all steps in the process.

- Once you have maximized the quantity of protein and have found the optimal SDS-PAGE conditions for maximum density of the final protein bands, run the sample under these conditions.

- Electroblot the sample to PVDF membrane, by whatever protocol you are most comfortable with.

- Stain the PVDF with Ponceau red. Amido black is a second choice stain (more aggressive), leaving coomassie blue as a poor choice for PVDF, but it can still be used with care.

- Wash the blot to remove excess stain. Generally the same solution as the stain without the coloring agent is fine to remove excess stain.

- Cut out the bands of interest tightly, leaving no unstained PVDF. Place then into a 1.5ml clear plastic snap-top micro Eppendorf tube.

- Rewet the PVDF with 1drop methanol, then wash the blots with 1ml reagent grade water and vigorous vortexing for 1 minute. Repeat this wash twice. Ø Remove all liquid and let blot air dry.

- Label tube(s), ship over packed (in a small box or other container) to prevent breakage or crushing of the sample tube. There is no need to ship on dry ice as long as the blots are dry.

- Include the Protein Sequence Analysis sample form, filled out completely, along with the sample. A hardcopy of the PO# you will be using to pay for the analysis should also be included.
- If this is a recombinant protein, please include the expected sequence, which will be used to align the final result into a more meaningful report if the data is heterogeneous.

- A minimum of 5 cycles can be run, with a maximum of ~30 amino acids expected for most cases.

- Solution samples can also be submitted; however salts and other buffer components must be eliminated for quality sequence data. Normally a detailed discussion will be needed in these cases.