Solutions for Your Research

Protein Analysis Service

N-terminal Protein Sequencing

Please, provide the protein molecular mass if known.

A) Sample in solution

Please, provide a vial containing the frozen purified protein.
Recommended buffer: aqueous solution or organic solvents (as Acetonitrile/TFA), please avoid the presence of primary and secondary amine (do not use Tris buffer), and avoid high concentration of detergents and Glycerol or non volatile salts.
Recommended amount: 50-300 pmol

B) Sample blotted onto PVDF membrane after SDS-PAGE electrophoresis

Best conditions for SDS-PAGE electrophoresis:
use the highest purity gel reagents possible, make fresh stock solutions, use reducing agents during electrophoresis, wear gloves.
The most useful concentration of acrylamide is 12.5%, use a mini-gel system (0.75 mm thick).
For samples and markers use "sample buffer" 1 X (10% glycerol, 5% b-mercaptoethanol, 3% SDS, 62.5 mM Tris pH 6.8, 0.025% bromophenol Blue).
Best conditions for electroblotting:
use only PVDF membrane for blotting procedures and please avoid Tris-Glycine buffer (recommended buffer is CAPS [3-Cyclohexylamino-1-propanesulfonic acid] 10 mM pH 11 and 10% Methanol).
After blotting, stain the gel in Coomassie-blue R-250 solution in water (0.25% w/v), 50% methanol, 10% glacial acetic acid for about 60 min. and destain in 25% methanol and 7% glacial acetic acid.
Remove the membrane and stain it in Comassie-Blue R-250 solution (0.25% w/v) in 50% methanol for about 5 min., wash it with water and destain it in 50% methanol with constant shaking, for about 5-10 minutes. Finally wash with deionized water.

Please, identify the sample of interest and send a copy of the gel or membrane image, marking the bands or spots of interest.
Visible spots stained with Comassie, contain at least 100 pmol of protein; please do not cut bands from the membrane, just send the intact membrane sandwich between 2 pieces of Whatman paper, at room temperature.