

N-terminal Sequence Analysis of Proteins Sample Preparation Guidelines

Sample Submission for N-terminal Sequence Analysis

Protein amount: A **minimum of 10 pmol** protein or peptide is required for routine analysis

Sample format:

- On pvdf membranes (Coomassie stained; silver stain not acceptable)
- In solution as concentrated as possible (volume must be 100-600 ul; for acceptable solvents see section D)
- Lyophilized

Turn around times: 5-10 business days

A. General sample handling

To minimize protein losses from adsorption to walls of tubes, use polypropylene tubes or silicized glass tubes. Avoid polystyrene (clear plastic) and untreated glass.

Use freshly prepared, high purity reagents and water. Contaminants from buffers, detergents, urea, guanidine can prevent a good sequencing result. Example: Old urea solutions can contain cyanate which reacts with the amino terminus of proteins and thus blocking the sequencing reaction.

ALWAYS WEAR GLOVES while preparing a protein sample.

The purity of the protein for Edman sequencing should be 85% or higher. Contaminating peptides or proteins interfere with the Edman chemistry and contribute to a higher noise level. **The lower the amount of protein, the higher the purity required.**

B. Gel electrophoresis

Use completely polymerized gels by either using precasted gels (i.e. Novex) or by pouring the separation gel the day before you plan on running the protein. Free acrylamide can react with the amino groups on your protein during polyacrylamide gel electrophoresis, which reduces the chances for good sequencing data.

For SDS-PAGE always run standard proteins or markers and a blank on the same gel. Protein quantity can be estimated by running the appropriate molecular weight markers in differing concentrations on the same gel as the sample.

C. Electroblothing on PVDF membranes

Determine how much protein you have on an analytical gel before you blot. Obtain standards of known concentrations from the facility. Then consult with us to see if you have enough protein before you blot you protein to PVDF. Count on a loss of 50-75% during blotting. You will need 10 pmol of protein on the PVDF.

Important: Use PVDF membranes only. Nitrocellulose trashes the sequencer.

Pre-Wet PVDF with 100% methanol for 30 sec followed by equilibration in transfer buffer for 5-15 min.

Common transfer buffers:

CAPS: 10 mM CAPS, 10% methanol, pH 11.0. Degas before use.
Tris-Glycine: 25 mM Tris, 192 mM Glycine, 20% methanol, pH 8.3.
CAPS buffer is preferred for sequence analysis (no glycine contamination).
If Tris-Glycine is used the membrane needs to be washed thoroughly with dd- water following transfer and staining. User bulletin on electroblotting and references are available.

D. Proteins submitted in solution

Submit at least 10 pmol of protein.

Sample volume must be between 100 and 1000 microliters

The following components are ACCEPTABLE:

Water/0.1 TFA

Water/0.1 M acetic acid

Sodium/potassium salts up to 0.2 M

PBS

Guanidine/HCl up to 7M

Acetonitrile and methanol less than 15%

The following components are NOT acceptable:

Ammonium buffers

SDS

Brij 35

Tris base

CHAPS

Triton X-100, reduced Triton X-100

DMSO

Tween

DMF

Zwittergent

Glycerol

any other detergent

Salts and buffers >100 mM

Sucrose