

N-terminal Protein Sequencing: Sample Requirements

Genosphere Biotechnologies

Bioanalysis Division

info@genosphere-biotech.com

Sample amount

We require a minimum 20 pmoles of blotted protein. Whenever possible, supply 50 pmoles, it usually generate unambiguous data.

| Protein size | µg for 20 pmoles |
|--------------|------------------|
| 10 kDa | 0.2 |
| 25 kDa | 0.5 |
| 50 kDa | 1.0 |
| 75 kDa | 1.5 |
| 100 kDa | 2.0 |
| 125 kDa | 2.5 |

Sample form

We accept PVDF blots or purified lyophilized/solubilized proteins.

Blotted onto a PVDF membrane (preferred). The protein should be electroblotted from 1D or 2D gels and stained with Coomassie Blue (R250). Following staining/destaining, blotted membranes should be rinsed thoroughly with deionized water. The sample should be as concentrated as possible (15 to 20 pmol / lane) and several gel lanes may be loaded and submitted. The whole membrane may be submitted with a copy showing the band(s) of interest or the bands may be cut out and submitted, please indicate band molecular weight.

Protein in solution or lyophilized. 30 to 150 µl of solution (H₂O, acetonitrile, propanol). Pure lyophilized protein is also acceptable. Protein should be free of detergent, salts and buffers. Please note that any amine-containing chemical present (e.g. Tris) will impair the adduction step. Extra charge applies for this type of sample as we will run pre-purification at nominal fee to ensure complete removal of amine-containing impurities.

Sample purity

PVDF blots: well separated bands.

Liquid: Single purified proteins. Immunoprecipitation and affinity-column purified usually give good samples for sequencing. In any case, a mandatory additional column purification step is applied to all incoming solution samples.