

Authorization for Protein/Peptide Sequencing

Principal Investigator: _____ Department: _____
 Contact Person: _____ Phone: _____
 Client Signature: _____ ISC#: _____
 Date received: _____ Date completed: _____

Billing address for off campus users: _____

Sample Description:

Sample I.D.: _____ Mol. wt: _____
 Amount (pmol): _____ Internal Sample I.D.: _____

Sample Features (check all that apply):

- | | | |
|---|---|--|
| <input type="radio"/> In salt free volatile liquid | <input type="radio"/> Dry with salts | <input type="radio"/> Contains modified residues |
| <input type="radio"/> In liquid with non-volatile salts | <input type="radio"/> On PVDF membrane | Modification _____ |
| <input type="radio"/> Dry with no salts | <input type="radio"/> For internal sequencing (see below) | |

Purification Methods Used (check all that apply):

- | | | |
|---------------------------------------|---|------------------------------------|
| <input type="radio"/> HPLC | <input type="radio"/> 2-D gel electrophoresis | <input type="radio"/> Ion exchange |
| <input type="radio"/> Electroblothing | <input type="radio"/> Affinity chromatography | <input type="radio"/> Other _____ |
| <input type="radio"/> Electroelution | <input type="radio"/> Gel filtration | |

Last Method of Purification: _____

Was Tris, glycine, or other amine buffers used in last or next to last step? Yes No

Reduction and alkylation required? Yes No

Number of residues to be analyzed: _____

Additional Comments/Instructions:

Internal Sequencing For Proteins With Blocked Amino Terminus:

Digestion protease (select one or a combination):

- | | |
|--|-----------------------------|
| <input type="radio"/> Modified trypsin | <input type="radio"/> Lys C |
| <input type="radio"/> Glu C | <input type="radio"/> Other |

Number of peptides to sequencer? _____ Number of residues to be analyzed per peptide? _____

(OVER)

Additional Comments/Instructions for Internal Sequencing:

Cautions and Recommendations:

- Samples containing non volatile salts, especially reactive amines such as Tris or glycine, require special treatment prior to sequencing. Please indicate above
- PVDF membranes from Schleicher and Schuell are not satisfactory and should not be used. Also, never submit nitrocellulose blots for direct sequencing.
- Use clean dishes for destaining and storing PVDF blots; Western blotting dishes are typically contaminated with milk proteins.
- Two proteins can be sequenced simultaneously if one sequence is known or if one is in greater excess of the other.
- Sequencing can be performed at low pmol levels, especially if the sample is homogeneous.
- When using immunoaffinity columns compare the mol. wt. of your unknown protein with that of immunoglobulin light or heavy chains. These columns can leak Ig chains.
- Albumin is a common contaminant from cell cultures and has a mol. wt. of ~68,000.
- Visit our Web Site at www2.utmb.edu/proch for blotting procedures and further information.

Protein/Peptide Sequencing Facility Personnel

Core Manager:
Steve Smith, M.S.
Email: jssmith@utmb.edu
Phone: (409) 772-6766
Fax: (409) 747-4753

BRF Assistant Director:
John E. Wiktorowicz, Ph.D.
Email: jowiktor@utmb.edu
Phone: (409) 772-2764
Fax: (409) 772-8025

Facility Director:
Alex Kurosky, Ph.D.
Email: akurosky@utmb.edu
Phone: (409) 772-2771
Fax: (409) 772-8025

Sequencing Charges

Protein/peptide Sequence Analysis on Procise cLc 494, or PROCISE 494HT
\$20.00 per residue (per Edman cycle) with a five residue minimum

Internal Sequence Analysis using the ABI 173A Microblotter
-\$250 per sample plus sequencing charges

Analysis includes enzymatic digestion of protein and sample blank, capillary HPLC, peptide isolation and collection of PVDF. Subsequently obtained peptide fractions are sequenced at the above rate.