N-TERMINAL PROTEIN SEQUENCING

CREATIVE PROTEOMICS

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Why Choose Creative Proteomics?

Creative Proteomics can provide N-terminal sequencing by Edman degradation or mass spectrometry (MS), with complementary advantages to each other. We can provide N-terminal sequence analysis of peptides, therapeutic proteins, monoclonal antibodies, and protein vaccines. In addition to high-quality results, you will also receive:

- High sensitivity, capable of sequencing at the low picomole level
- Rapid return of results
- Clear and understandable reports
- Expert advice from our highly trained staff

Applications

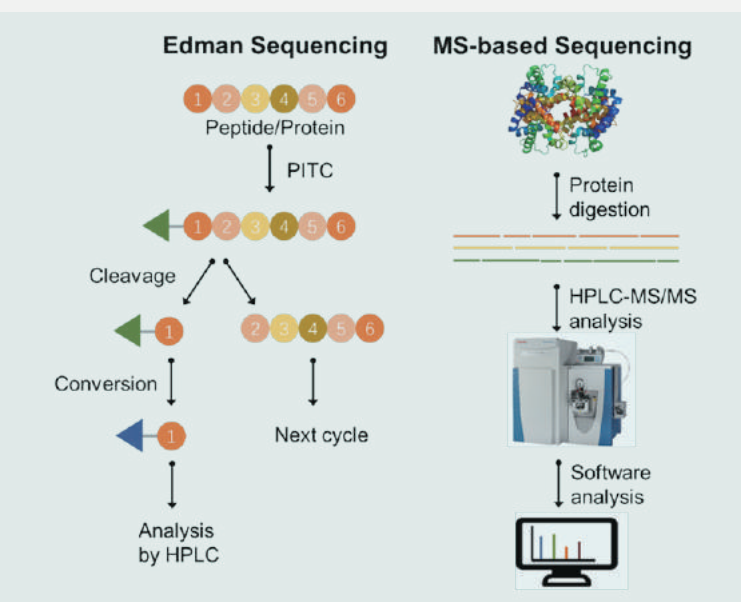
- Determination of the N-terminal amino acids of a protein or peptide.
- Identification and characterization of proteins or peptides.
- Confirmation of the sequence, purity, and correct translation of a recombinant protein.
- Identifying proteins isolated from species where most of the genome has not yet been sequenced.
- Mapping modified residues or crosslinked sites in proteins that prove to be refractory to analysis by mass spectrometry.
- Quality control of proteins according to ICH Q6B Guideline.

The N-terminus is the start of a protein or polypeptide. It influences the subcellular distribution, degradation, and the turnover rate of a protein. Therefore, the N-terminal sequence analysis of a protein is important for protein function study. In addition, in pharmaceutical proteins, there are some often observed variations of N-terminus, including incomplete processing of the N-terminal signal sequences, formation of pyroglutamate, and the lack of removal of the N-terminal methionine residue. Therefore, N-terminal sequencing is an important quality control analysis. The N-terminal sequence confirmation of drug substance is also required by the ICH Q6B.

Methods of N-terminal Sequencing

Edman sequencing: The Edman sequencing reaction is a cyclic procedure, in which N-terminal amino acid residue is repeatedly cleaved off and the removed amino acids are identified by chromatography. The coupling and cleavage reaction efficiency is not 100% and in practice limits the number of residues that can be sequenced less than 50 residues.

Mass spectrometry technique: In MS-based sequencing, proteins are pretreated with digestive enzymes to be digested into small fragments, which are analyzed by mass spectrometers. Since most amino acid residues have different mass values, different peptides can produce fragment ions with different m/z ratios and different spectra. Theoretically, the amino acid sequence of the peptide can be figured out from the MS/MS spectrum.



Key Features

Edman sequencing and MS-based sequencing have their own features. Edman sequencing can accurately analyze N-terminal amino acid sequence, especially discrimination of isoleucine/leucine, and glutamine/lysine. Our protein sequencer system automates the Edman reaction, HPLC separation and detection, and data analysis. For MS-based N-terminal sequencing, we can analyze the N-terminal blockage and post-translational modifications (PTMs) by high-resolution mass spectrometers. And we can use a combination of 2 or 3 different proteases to increase the sequence coverage.

Technique	Edman Sequencing	Mass Spectrometry
N-terminal sequence	Yes	Yes
C-terminal sequence	No	Yes
Disadvantages	• Not available for the peptide whose N-terminus has been chemically modified or blocked. • Low throughput analysis.	• Isobaric amino acids are not determined. • The peptide sequence coverage is rarely 100%.
Advantages	• Identify the exact N-terminal amino acid, especially for isoleucine/leucine, and glutamine/lysine. • Directly analyze amino acid information without requiring any protein database. Therefore, it can identify unknown proteins that are not registered in databases.	• Obtain longer information of N-terminal sequence. • Analyze N-terminal blockage and PTMs.

Sample Preparation Guideline

Edman Sequencing

Proteins can be submitted in solution, as an SDS or native polyacrylamide gel piece, or blotted to PVDF membrane.

- In solution

  Samples should be free from interfering buffers, salts and detergents. If interfering buffers/salts are unavoidable, it's still possible to sequence the material after clean up. Amines (such as Tris, ethanolamine, and glycine) in the buffer should be avoided.
- In gel

  The gel slices of interest are cut out and sent in a microcentrifuge tube. Proteins can be eluted from polyacrylamide. Gels should be stained with Coomassie Blue R-250 or G-250 instead of use silver stain.
- On PVDF Membrane

  Proteins separated by SDS-PAGE should be blotted onto PVDF membrane. The protein can be stained with Coomassie Blue R-250, Amido black, Ponceau S or Sypro Ruby (Sliver stain is not recommended).

Note: Please feel free to contact us for detailed sample requirements.

MS-based Sequencing

Gel and liquid protein samples are acceptable and the sample purity should be as high as possible. Mass spectrometry is a much sensitive technology, and thus, samples must be prepared carefully. They can be subject to contamination of the sample, for instance, due to incomplete subcellular fractionation or purification of a multiprotein complex, overwhelming of the sample by highly abundant proteins, and contamination from skin or hair. Any source that may introduce contaminating proteins should be eliminated.

You will receive

Results are delivered in a detailed and understandable report, including

- Experiment procedures
- Instrument parameters
- Raw and processed data
- A summary of the sequence analysis

Related Services for Protein Identification

- Peptide Mass Fingerprinting (PMF)
- Membrane Protein Identification
- *De Novo* Peptides/Proteins Sequencing Service
- *De Novo* Antibody Sequencing

Contact Us