

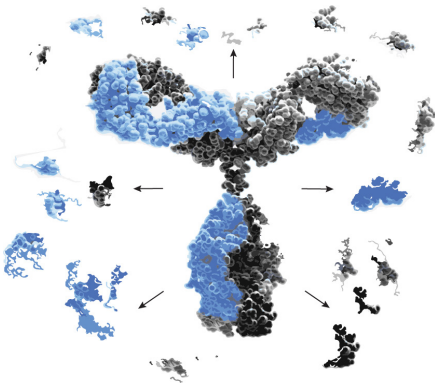
# De Novo Sequencing

## Protein/Peptide De Novo Sequencing by Mass Spectrometry

De novo sequencing is the process of determining the amino acid sequence of a protein based directly on the fragments detected in the mass spectrometer. This sequence information from a protein is important in order to study the biological function or to confirm proper identification of a product. Previously, protein sequencing was done by Edman degradation using peptide sequencers. However, this process has now been superseded by mass spectrometry due to the speed and accuracy of the instruments now available.

## Monoclonal Antibody Sequencing

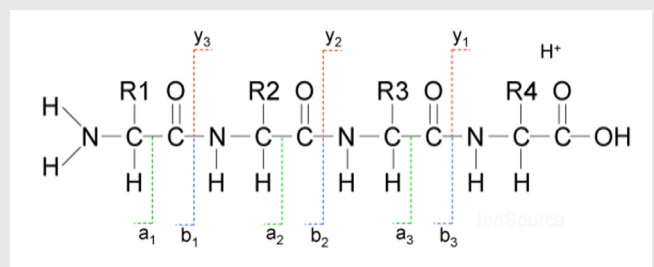
De novo sequencing is often used to determine the full amino acid sequence from a monoclonal antibody. This technique can fully identify, to the single amino acid resolution, both the light and heavy chains from a provided antibody sample using multiple enzymes for digestion.



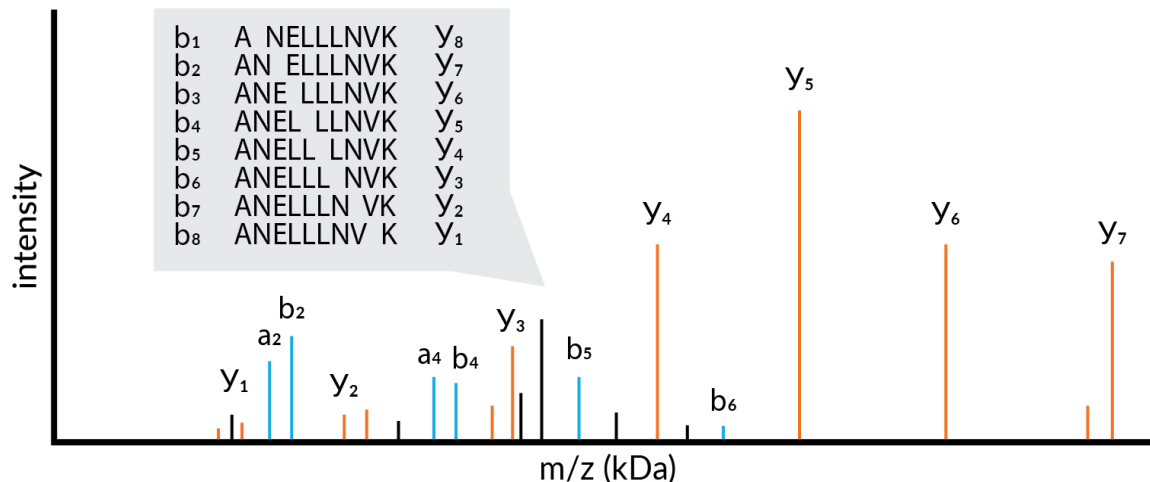
## Notable Benefits with CovalX De Novo Services

- **Expertise:** CovalX has over 15 years of experience characterizing biotherapeutics using mass spectrometry.
- **Latest Equipment:** CovalX utilizes the latest mass spectrometric instrumentation and analysis software available.
- **Trusted Company:** CovalX has worked with nearly all of the major Biopharma companies and specializes with smaller biotech as well.

Using special computer algorithms, this analysis can interpret and assign the sequences without prior database knowledge from the protein directly. De novo sequencing is often used for biotherapeutics development, bio-similar antibody characterization, or resolving ambiguity from gene sequencing of the monoclonal antibody.



Using MS/MS fragmentation, the most common peptide fragments observed in the mass spectrometer are the a, b and y ions, as described in the figure above.



The spectra of the fragment ions of each peptide can be more rapidly and accurately interpreted and assign the sequences without prior database knowledge of the proteins sequence.

# De Novo Sequencing

## How it works

After sample receipt, if necessary, the sample can first be purified using electrophoretic gel. Then, the protein to sequence is digested using seven different proteolytic enzymes analyzed separately and in replicate to ensure the highest possible sequence coverage. The peptides are then introduced into the mass spectrometer after nano-flow liquid chromatography separation. The mass spectrometer measures not only retention time and intact mass from the peptides, but conducts MS/MS fragmentation inside the

mass spectrometer to create fragment ions from each of the peptide digests. This MS/MS measurement provides the highest possible sensitivity and accuracy of peptide detection and identification available.

The analysis software can accurately assign amino acid sequences without any prior knowledge from the protein using special algorithms. Our high-resolution nano-LC coupled with ultra high-resolution mass spectrometry detection is finely tuned to produce unmatched quality in antibody sequencing.

## De Novo Sequencing: How It Works

