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FlashNote

# Rapid Top-Down Analysis of the NISTmAb antibody

**Protein sequence verification by LC-MS peptide mapping is time consuming. An enhanced method to verify the sequence of recombinant therapeutic proteins and reagents can improve the productivity of labs that need to verify large numbers of proteins. Intact protein sequencing by top-down mass spectrometry is a time-efficient and straightforward solution to address these requirements. A simple LC- free MALDI top-down method with fast protein reduction is presented to deliver an N/C terminal read of the NIST antibody light and heavy chain.**

## Challenge

With more than 50 products being approved for use in disease and 50 more investigated in advanced clinical trials, monoclonal antibodies (mAbs) and related products are the fastest growing class of human therapeutics. Confirmation of known sequences or identification of mutation sites, clips or residual signal peptides is essential since the primary sequence and associated PTMs impact the potency of these molecules. Methods are needed to examine these aspects with rapid and robust performance. In quality control applications for biotherapeutics with given sequences, high throughput screening is also desired.

## Solution

A simple and rapid MALDI top-down method can elucidate the modification status of the N- and C-termini of a protein. In addition, the sequences of proteins up to approx. 25 kDa can be completely verified in minutes using this approach. Fragments from much larger molecules such as mAbs after reduction and/or specific enzyme cleavage can be readily characterized and verified. MALDI top-down data will be sent for analysis in BioPharma Compass 3.1 software.

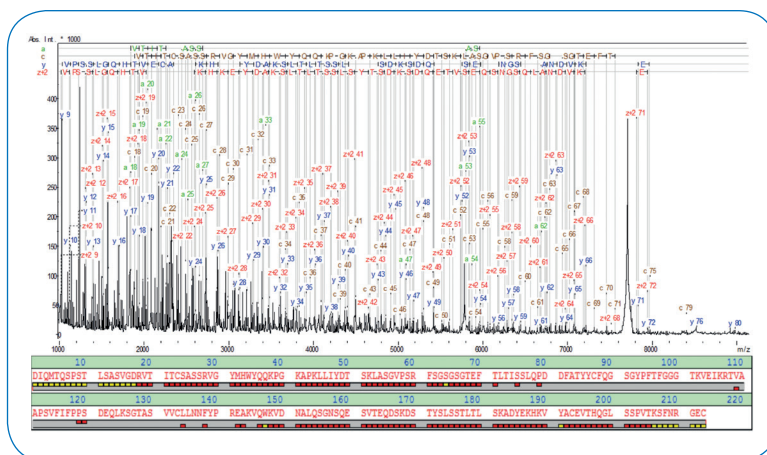


*Figure 1: Top-down sequencing analysis of NISTmAb light chain. 70% sequence coverage is achieved and no sequence error or unexpected modifications are detected in the covered mass range.*

# Start Saving Time!

## Your sequence verification of protein therapeutics can be done in such a rapid and robust way!

### Materials

- 10 mg/mL NISTmAb, humanized IgG1k monoclonal antibody (RM8671) in its formulation buffer
- 0.1% trifluoroacetic acid (TFA) in water
- Acetonitrile (ACN)
- 1 M Dithiothreitol (DTT) in water
- 8 M Guanidin-HCl in water
- 25 mg/mL sDHB (Bruker #8209813) in 50% ACN/0.1% TFA in water
- Amicon Ultra-0.5 centrifugal filter unit, 10 kDa (Millipore #UFC5010)
- Bruker MTP BigAnchorChip sample plate (Bruker #8280788)

### Method

- Add 8.5 µl 8 M Guanidin-HCl and 1.5 µl 1 M DTT to 5 µl of NISTmAb solution
- Incubate the mixture for 1 h at 37°C
- Purify the reduced sample at least 2x using the Amicon cut-off filters according to manufacturer's manual
- Add 2 µl of the purified sample to a BigAnchor plate spot and incubate for 3 min (don't let it completely dry)
- Add and remove 10 µl of 0.1% TFA to wash the anchor spot twice
- Add 0.5 -1 µl of sDHB matrix solution to the anchor spot and let it dry

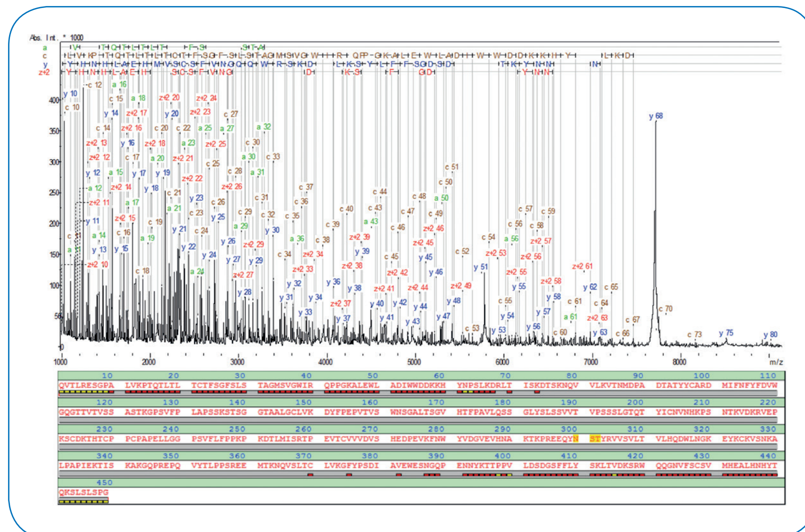

*Figure 2: Top-down sequencing analysis of NISTmAb heavy chain. Matching N-terminal fragments confirm the N-terminal pyroGlu modification and the matching C-terminal fragments confirm the truncation of C-terminal Lysine.*


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