Protein Molecular Mass Measurement

By Intact Protein Analysis Using Mass Spectrometry

Introduction

Intact protein analysis provides information on the accurate mass of the protein and the relative abundance of its isoforms and modifications. It is an invaluable approach for the analysis of biologics throughout manufacturing to assess purity and heterogeneity characteristics for lot release purposes.

Poochon's intact protein analysis services are designed for intact protein mass measurement to confirm the sequence of a purified protein and the relative abundance of its isoforms, for example modifications with different glycans. Intact protein characterization is performed on samples in solution using a combination of liquid chromatography (LC) and electrospray ionization mass spectrometry (ESI-MS). Purified proteins with a molecular weight up to 160 kDa at a minimum amount of 1 µg have been analyzed successfully.

Specifications

→ **Method** – Intact protein mass spectrometry analysis

→ **Key Instruments** – Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, Orbitrap Exploris™ 240 Mass Spectrometer & 480 Mass Spectrometer

→ **Mass Specification** – Mass Accuracy: < 20 ppm; Mass Range: 40 to 8000 m/z

→ **Acceptable Samples** – Purified protein, in solution or dried (≥10 µg/sample, purity ≥90%)

→ **Turnaround Time** – Typically, reports are available within 5 business days of sample receipt

Schematic of Procedure Workflow

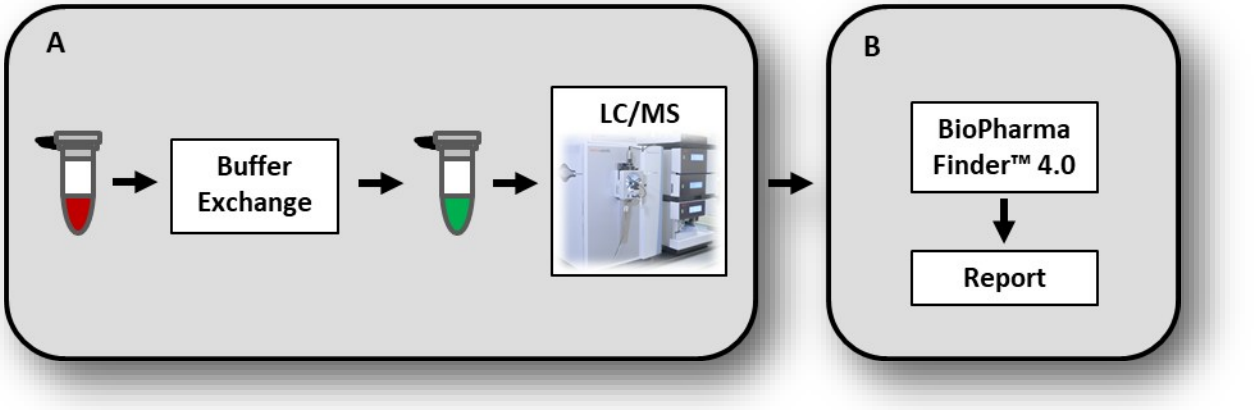

Figure 1:

A) Workflow for intact protein mass spectrometry analysis. *Note: LC/MS = liquid chromatography and mass spectrometry.*

B) Bioinformatic analysis approach used for mass analyses of datasets.

Glycan Forms Analysis of NIST mAb RM8671

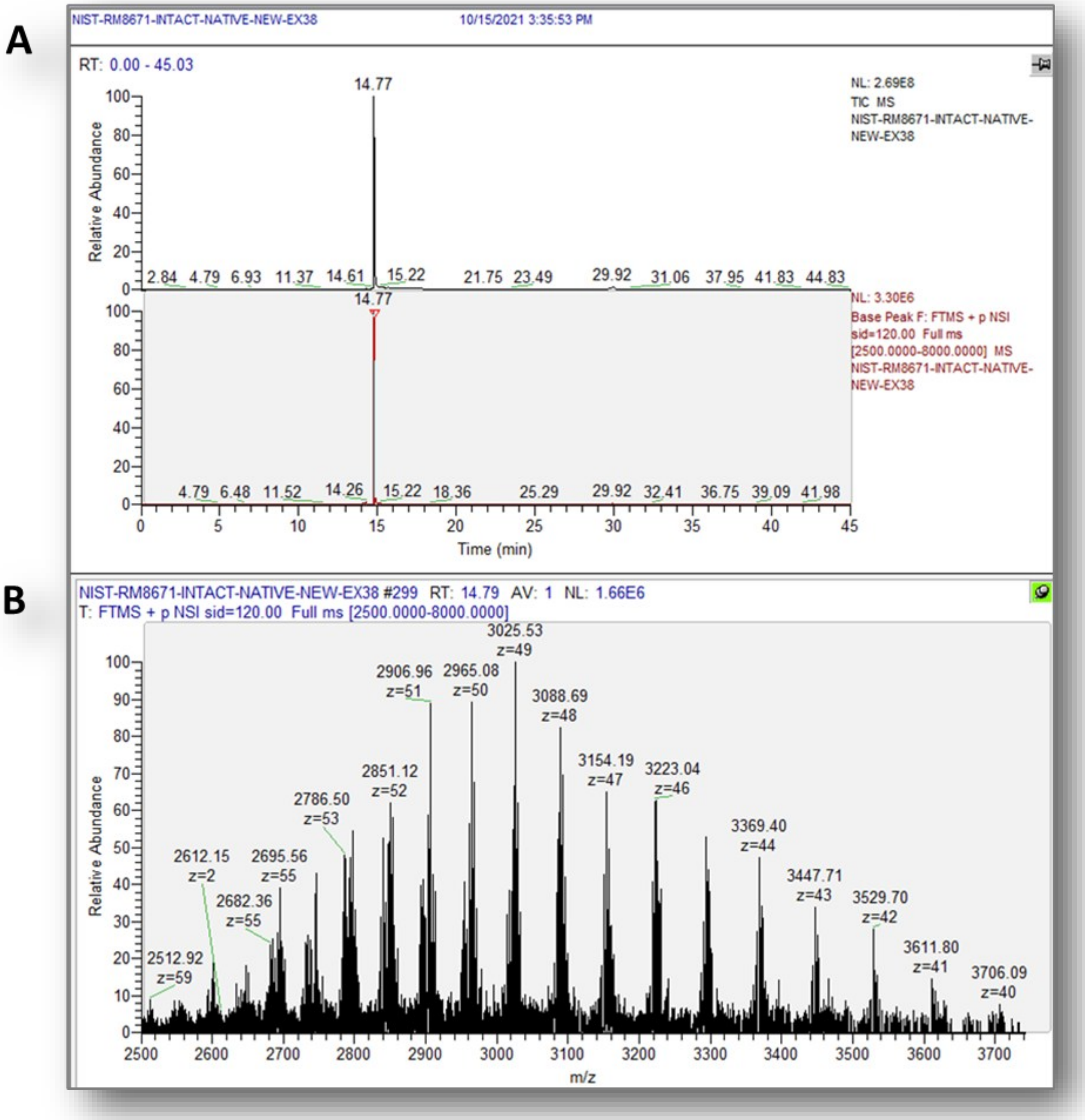

Figure 2: Intact mass measurements of the NIST mAb using liquid chromatography-mass spectrometry (LC-MS). Raw mass spectrum of intact NIST mAb using an Thermo Exploris 240 with protein mode mass spectrometer. **A)** Chromatogram, peak is the native intact NIST mAb RM 8671. **B)** Full MS spectra of intact mAb from the peak.

Glycan Forms Analysis of NIST mAb RM8671

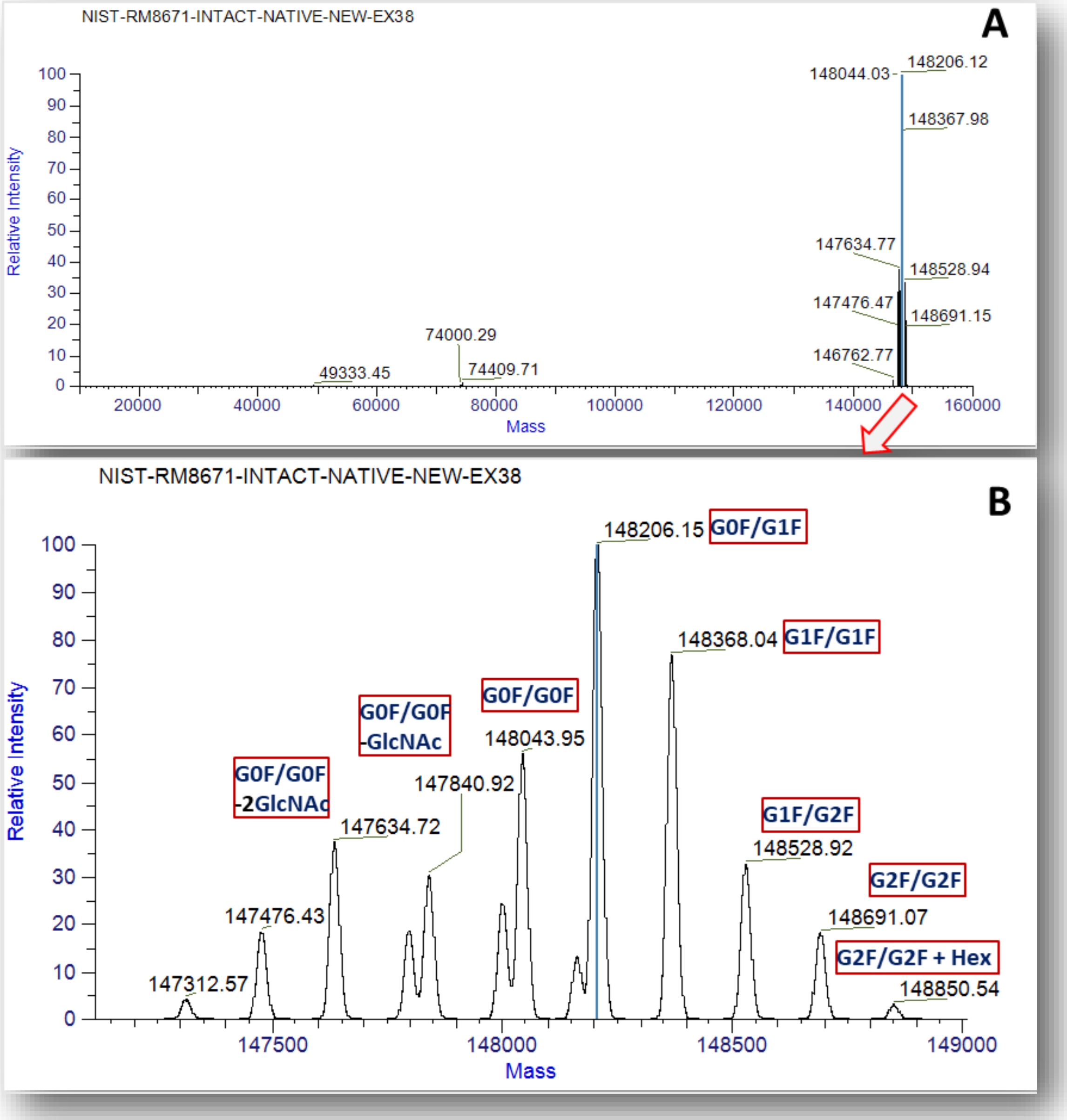

Figure 3: Intact mass measurements of the NIST mAb using liquid chromatography-mass spectrometry (LC-MS). **A)** Deconvoluted Spectrum range from 10 kDa to 160 kDa showing the high purity of the sample. **B)** Deconvoluted Spectrum range from 140 kDa to 150 kDa (enlarged from A) showing the glycan forms detected.

LC and HC Analysis of NIST mAb RM8671

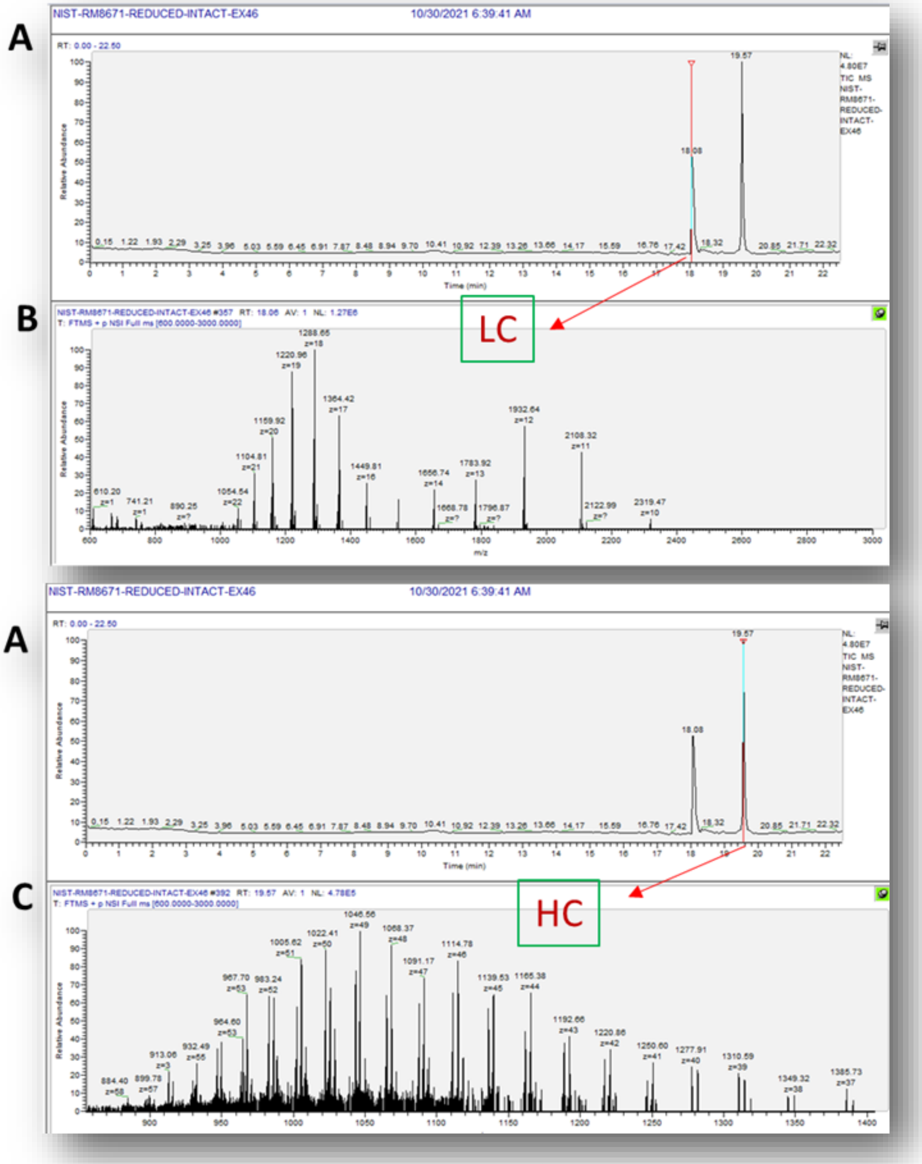

Figure 4: Intact mass measurements of the NIST mAb using liquid chromatography-mass spectrometry (LC-MS). Raw mass spectrum of intact reduced NIST mAb using Thermo Exploris 240 with protein mode mass spectrometer. **A)** Chromatogram, two peaks are the reduced intact NIST mAb RM 8671 Light Chain and Heavy Chain; **B)** Full MS spectra of LC from the corresponding peak. **C)** Full MS spectra of HC from the corresponding peak.

LC and HC Analysis of NIST mAb RM8671

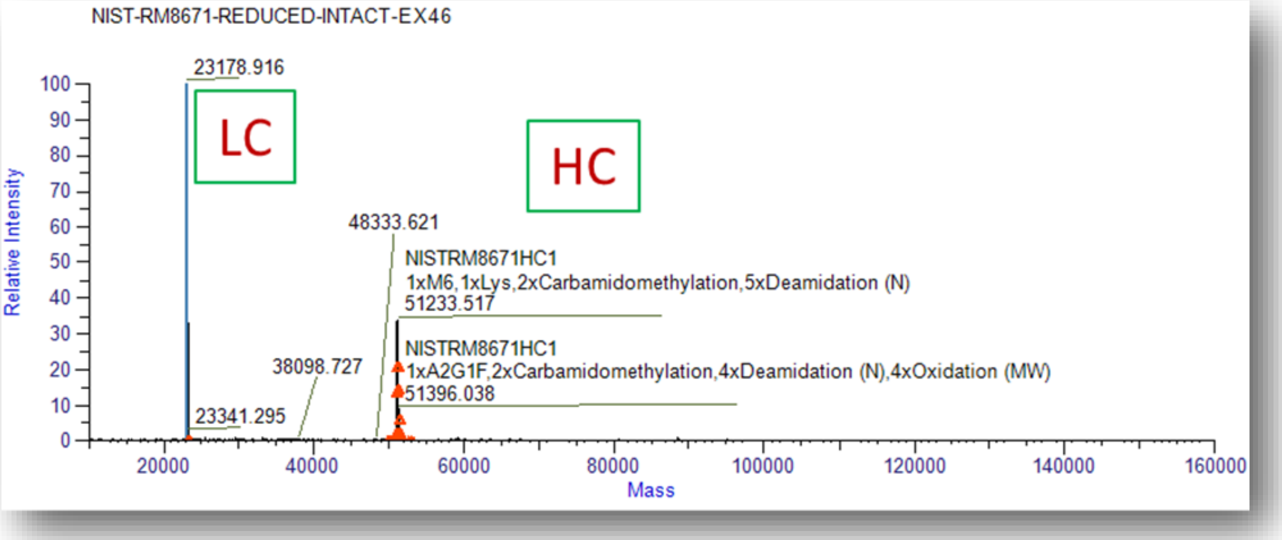

Figure 5: Intact mass measurements of the reduced NIST mAb using liquid chromatography-mass spectrometry (LC-MS). Deconvoluted Spectrum range from 10 kDa to 160 kDa showing the LC and HC molecular mass determined.

DAR Analysis of mAb ADC Sample

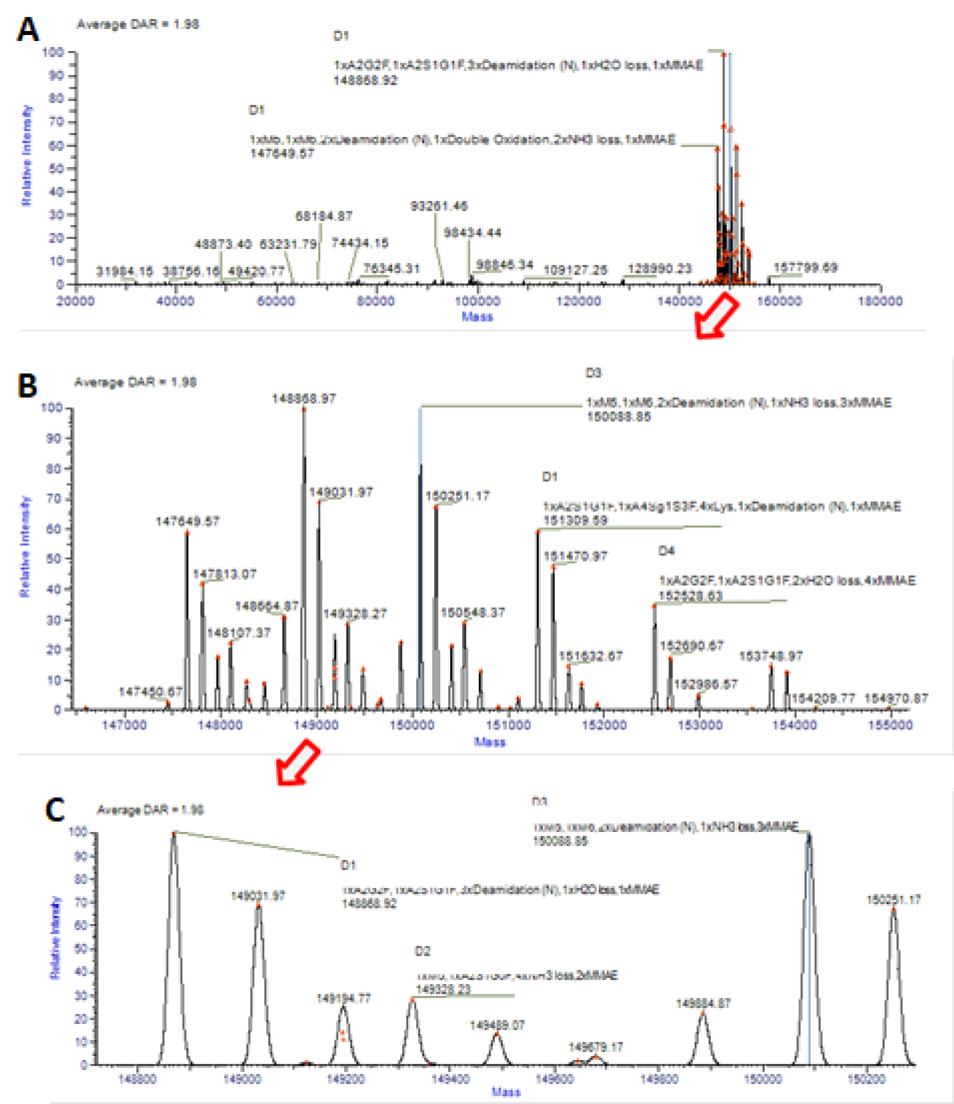

Figure 6: Deconvoluted Spectrum from intact protein analysis of one native mAb ADC sample. **A)** Deconvoluted Spectrum range from 20 kDa to 180 kDa showing the high purity of the sample. **B)** Deconvoluted Spectrum range from 140 kDa to 155 kDa (enlarged from A) showing the major forms of lysine-linked ADCs (MMAE) and glycan forms detected. **C)** Deconvoluted Spectrum range from 148 kDa to 151 kDa (enlarged from B) showing the major forms of lysine-linked ADCs (MMAE) and glycan forms detected.

Intact Analysis of a Purified Recombinant Protein With His-tag

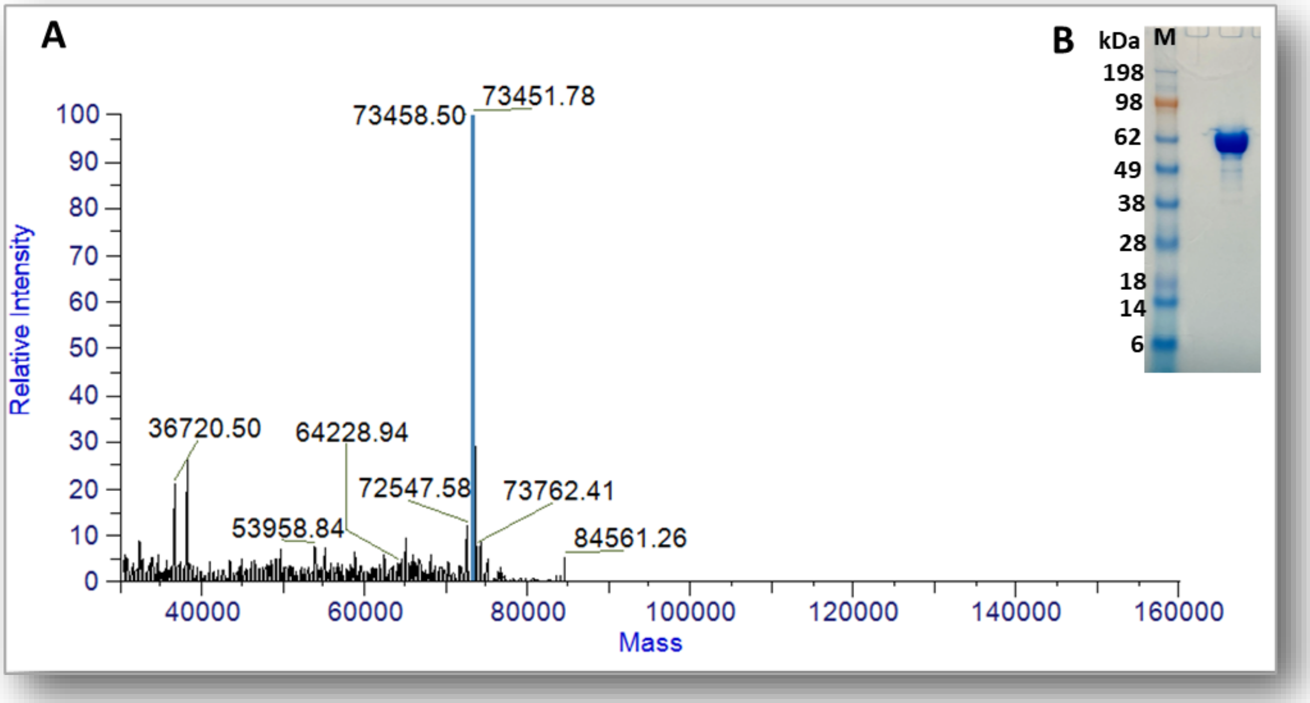

Figure 7: Deconvoluted Spectrum from intact protein molecular mass measurement analysis of native his-tagged recombinant protein sample. **A)** Deconvoluted Spectrum range from 30 kDa to 160 kDa showing the major components and the purity of the sample. **B)** *Insert:* Separation of the purified recombinant protein with his-tag sample (6 µg) on a 4-12% NuPAGE gel stained with SimplyBlue.