

# Hyphenating Size Exclusion Chromatography to High Resolution Mass Spectrometry - NIST mAb

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## Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. The hyphenation of SEC to high resolution mass spectrometry (HRMS) has enabled characterization of biologics. This non-denaturing, so-called “Native MS” is especially useful for higher order structure (HOS) of monoclonal antibodies (mAbs).

In this application, we demonstrate the use of SEC-HRMS for the characterization of NIST mAb RM 8671, a commonly used standard monoclonal antibody surrogate for method development. **Figure 1** shows the total ion chromatogram. Good peak shape is observed, and the low molecular weight fragment is separated even using a relatively short column (150 mm). **Figure 2** shows the deconvoluted spectrum for the main peak. Glycoforms are identified with good mass accuracies, with most glycoforms less than 10 ppm.

## LC Conditions

**Column:** Biozen™ 1.8 µm dSEC-2, 200 Å  
**Dimensions:** 150 x 2.1 mm  
**Part No.:** [00F-4787-AN](#)  
**Mobile Phase:** 200 mM Ammonium Acetate  
**Flow Rate:** 90 µL/min  
**Injection Volume:** 2.5 µL  
**Temperature:** 25 °C  
**Detection:** HRMS  
**Sample:** NIST mAb RM 8671, 10 mg/mL

**Figure 3** shows the deconvoluted spectrum for the low molecular weight aggregate, including putative full-length antibody minus the Fab arm.

In summary, SEC-HRMS is a powerful technique that can provide valuable characterization data, including confirming primary sequence and glycoforms, as well as confirmation of dimer and other high molecular weight species.

**Figure 1. Total ion chromatogram (TIC) for NIST mAb RM 8671, 25 µg**

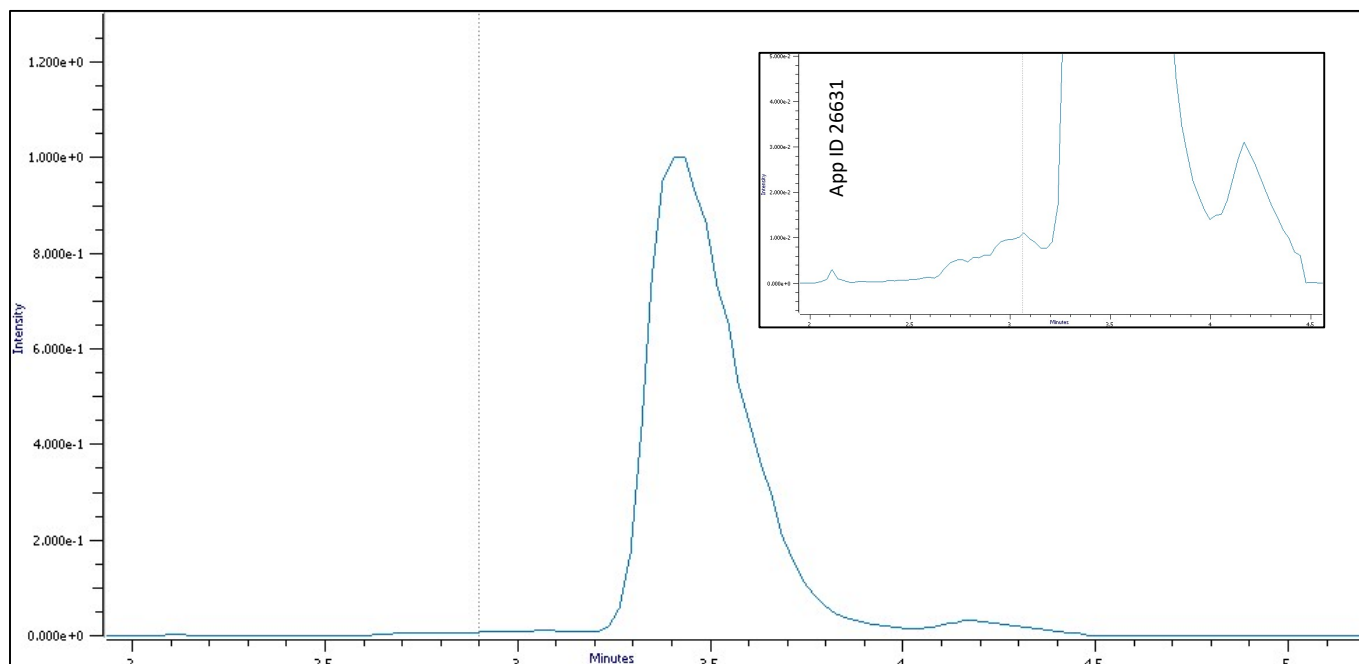


Figure 2. Deconvoluted Spectrum, Main Peak- NIST mAb RM 8671

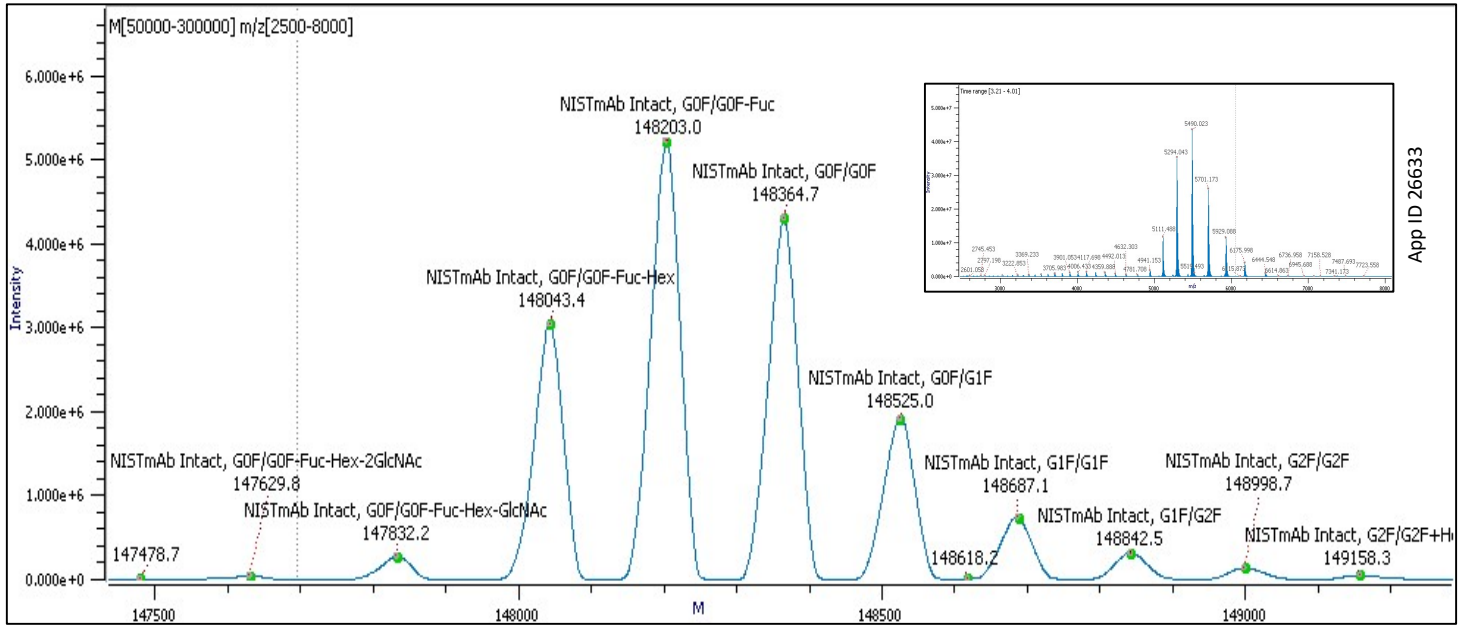
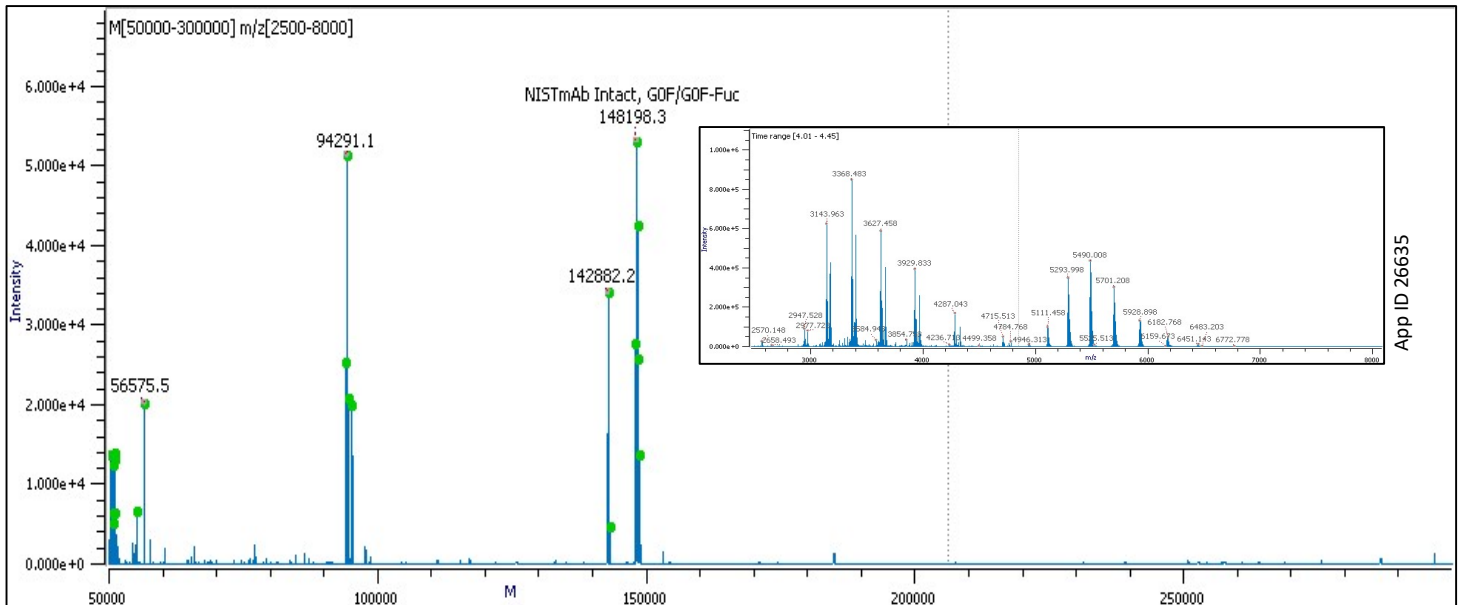


Figure 3. Deconvoluted Spectrum, Low Molecular Weight Fragment



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