

APPLICATIONS

Native MS Aggregate Analysis of NIST mAb using a bioZen™ 1.8 µm SEC-2 Column

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Overview

Monoclonal antibodies (mAbs) are widely used as therapeutics for the treatment of many diseases including cancer, inflammatory diseases, and cardiovascular diseases. As with all therapeutic proteins, mAbs are susceptible to degradation during production or storage leading to aggregation, fragmentation, and other post translational modifications. Monitoring aggregation is essential for therapeutic proteins as this can lead to decreased drug efficacy and increased immunogenicity. Characterizing a protein during drug development for protein aggregation is an essential part of any development workflow.

Native MS seeks to introduce molecules into the gas phase whilst retaining the conformations and interactions from solution. In native MS, only surface accessible sites are available to pick up charges resulting in an appearance at a high m/z ratio. When using native MS, source conditions must be maintained to remove buffers in tandem with retaining these fragile interactions that form tertiary structure of the protein. By using this in tandem with SEC, buffer exchange is not required prior to analysis as any buffer components which may suppress protein signal are resolved from the protein peak through size differentiation.

In this application note we look at a method for determining the level of aggregation in a commercially available sample of intact NIST mAb using a bioZen SEC-2 column together with the SCIEX X500B mass spec. 2 µL of intact NIST mAb was injected onto a bioZen 1.8 µm SEC-2 at a concentration of 10 µg/µL. Care was taken during injection to ensure the minimum sample volume is injected onto the column to prevent peak broadening, in addition tubing dead-volume should be minimized. The bioZen SEC-2 column gave good separation under conditions compatible with MS with the dimer clearly resolved from monomer in the total ion chromatogram (TIC). Fragment peaks were also clearly visible. The dimer was found to represent 0.12% of the monomer with fragment peaks also identified and confirmed by MS. The principle shown in this application note is the NIST mAb monomer presenting in the charge states of 26+ to 32+. The charge state envelope is indicative of a protein in a folded conformational state.


LC Conditions

Column: bioZen 1.8 µm SEC-2
Dimension: 150 x 4.6 mm
Part No.: [00F-4769-E0](#)
Recommended Guard: SecurityGuard™ ULTRA
Guard Cartridge Part No.: [AJ0-9850](#)
Guard Holder Part No.: [AJ0-9000](#)
Mobile Phase: 100 mM Ammonium Acetate
Flow Rate: 200 µL/min
Temperature: 25 °C
Detector: QTOF (SCIEX® X500B)
Sample: NIST mAb

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Need a different column size or sample preparation format?

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