

Size Exclusion Analysis Following IdeS Digestion Using a Biozen™ dSEC-2 Column

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Overview

Size Exclusion Chromatography (SEC) is a powerful chromatographic technique that allows for the highly selective separation of biomolecules and relies on the hydrodynamic radius of the analyte. In this application note, we demonstrate the analysis of antibodies using enzymatic digestion. Specifically, we use the cysteine protease IdeS, which cleaves IgGs below the hinge region and yields a Fab'2 & two Fc/2 fragments. These are easily segregated using the dSEC stationary phase. The below figures demonstrate the characterization of subunits of three monoclonal antibodies, post digestion with IdeS, using the Biozen dSEC-2 size exclusion column.

LC-UV Conditions

Column:	Biozen 1.8 µm dSEC-2
Dimension:	300 x 4.6 mm
Part No.:	00H-4787-E0
Mobile Phase:	200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH 6.2
Flow Rate:	0.35 mL/min
Injection Volume:	2.5 µL
Temperature:	25 °C
Detection:	UV @ 280 nm
Sample:	1. Trastuzumab (10 µg/µL) 2. Cetuximab (10 µg/µL) 3. Emicizumab (10 µg/µL)

Figure 1. Intact Trastuzumab Successfully Separated from IdeS Digested Trastuzumab Fragments, Fab'2 and Fc/2 Using the Biozen 1.8 µm dSEC-2 Column

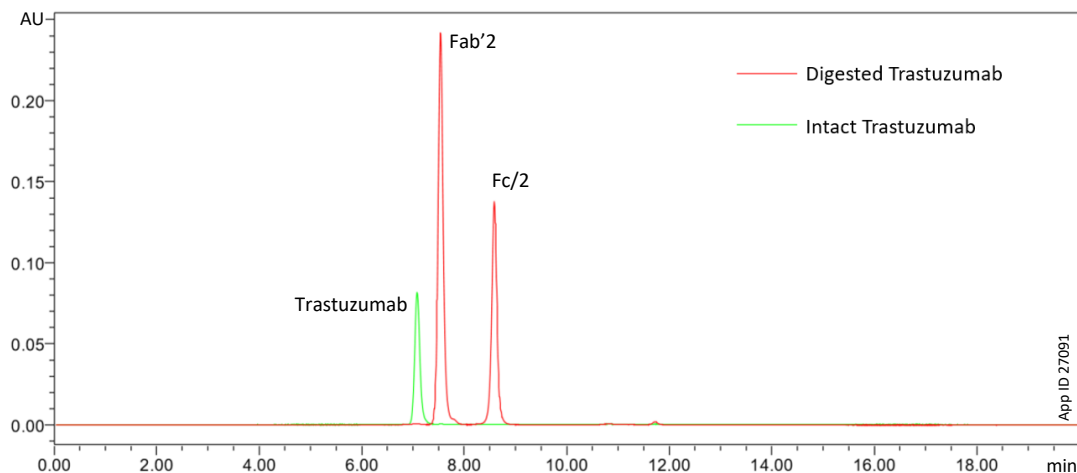


Figure 2. Intact Cetuximab Successfully Separated from IdeS Digested Cetuximab Fragments, Fab'2 and Fc/2 Using the Biozen™ 1.8 µm dSEC-2 Column

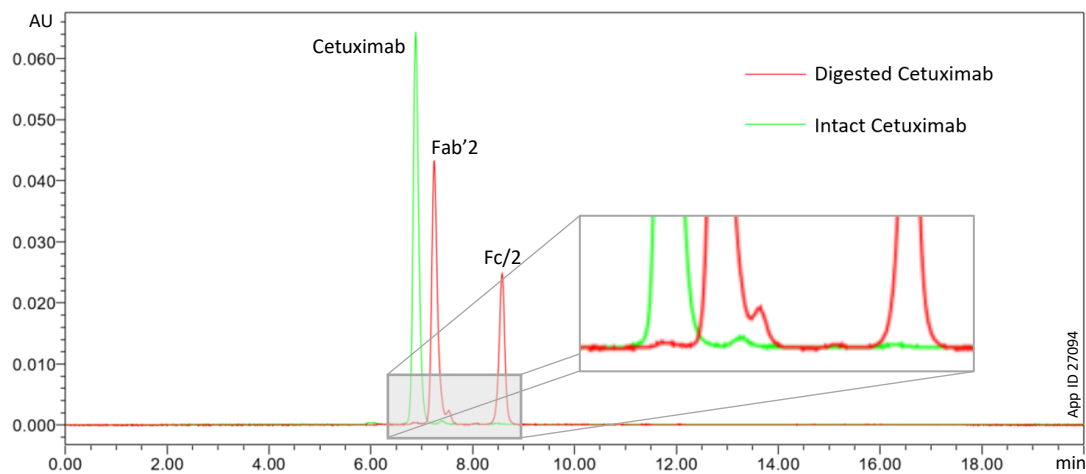
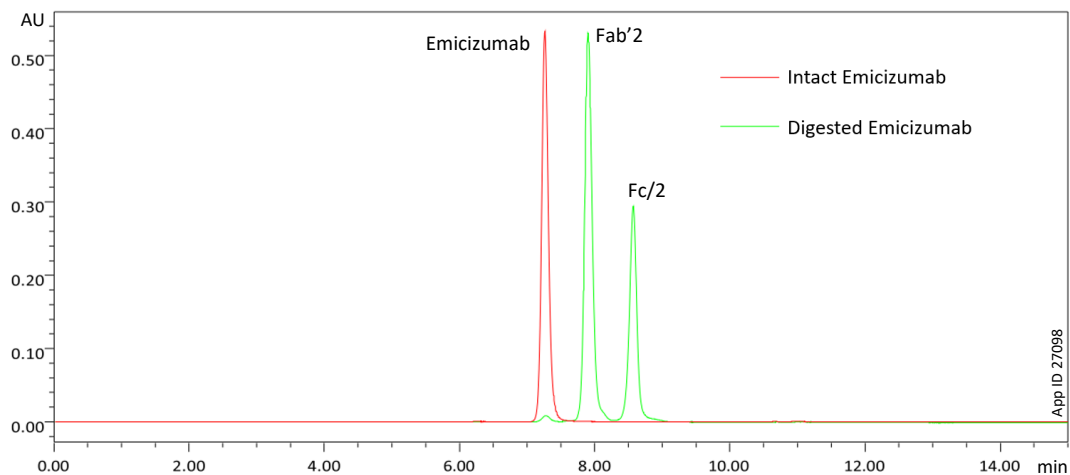


Figure 3. Intact Emicizumab Successfully Separated from IdeS Digested Emicizumab Fragments, Fab'2 and Fc/2 Using the Biozen 1.8 µm dSEC-2 Column



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