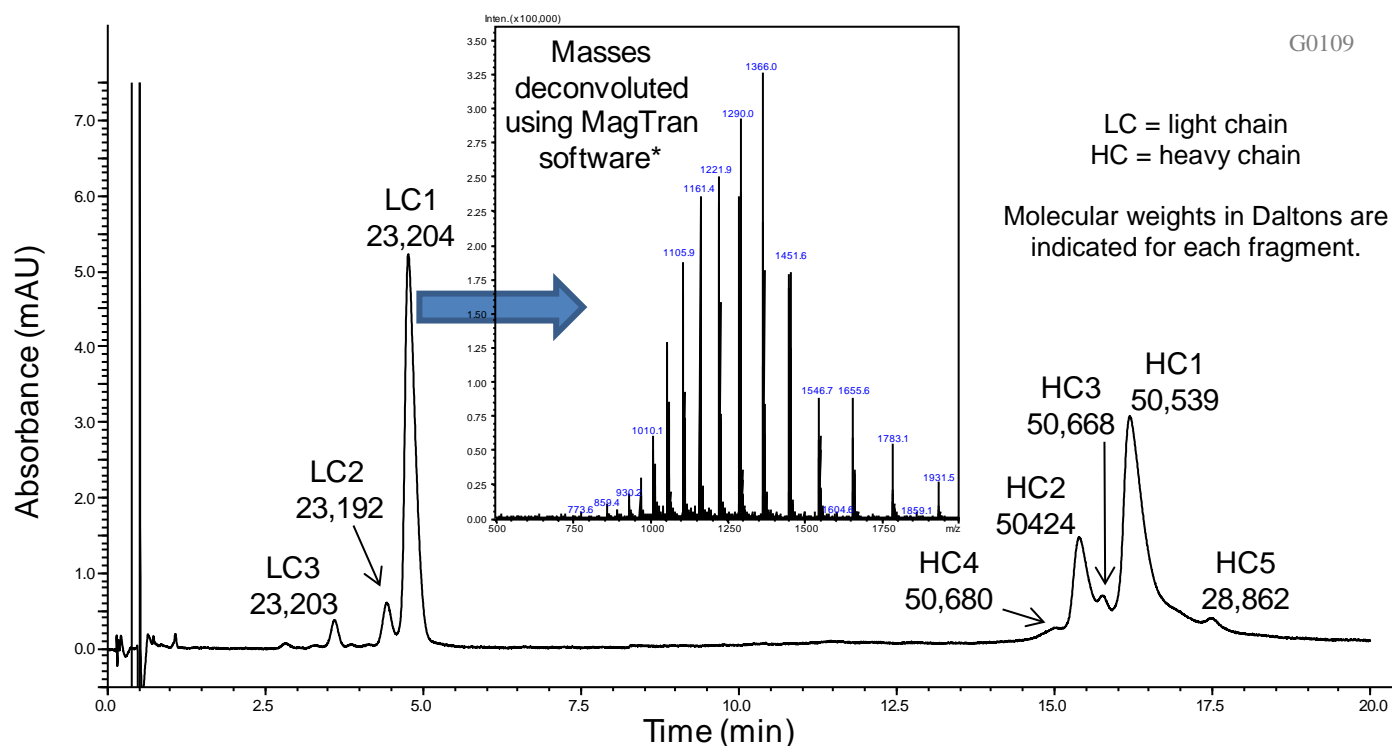


Application Note: 125-PR

## LC-MS Analysis of Reduced IgG1 Monoclonal Antibody Fragments Using HALO Protein C4



### TEST CONDITIONS:

Column: 2.1 x 100 mm, HALO Protein C4, 3.4  $\mu$ m  
 Part Number: 93412-614  
 Mobile Phase A: 0.5% (v/v) formic acid with 20 mM ammonium formate  
 Mobile Phase B: 45% acetonitrile/45% isopropanol/0.5% (v/v) formic acid/9.5% water with 20 mM ammonium formate  
 Gradient: 29-32% B in 20 min  
 Flow Rate: 0.4 mL/min.  
 Pressure: 20 bar  
 Temperature: 80°C  
 Detection: 280 nm and MS using 2 pps scan rate from 500 to 2000 m/z  
 Injection Volume: 2  $\mu$ L of 2  $\mu$ g/ $\mu$ L reduced and alkylated IgG1  
 Sample Solvent: 0.25% (v/v) formic acid in water  
 MS parameters: Positive ion mode, ESI at +4.5 kV, 400°C heat block, 225°C capillary  
 LC-MS System: Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

HALO Protein C4 has the low pH and high temperature stability that is required to analyze reduced and alkylated IgG1 using MS-compatible mobile phase. The use of 80 °C enables improved peak shape while the high resolution MS allows complete analysis of the IgG1 fragments that are present.

Adapted from *J. Chromatogr. A* 1315 (2013) 118-126.

\*Z. Zhang, A.G. Marshall, *J. Am. Soc. Mass Spectrom.* 9 (1998) 225.