Separation of Monoclonal Antibody (mAb) Monomer from its Half-body using Size Exclusion Chromatography

TSKgel
APPLICATION NOTE

Introduction

Monoclonal antibody (mAb) research continues to grow in an effort to develop effective biotherapeutics for a wide range of diseases. Recent research has shown an interest in mAb half-bodies as therapeutic vectors as they can be further targeted for conjugation, enzyme labeling, or antibody immobilization. Monoclonal antibody half-bodies can be generated through the genetic engineering of cells or by selective reduction of hinge-region disulfide bonds present in the mAb by mild reducing agents, such as TCEP [tris(2-carboxyethyl)phosphine]. Due to its lack of odor and resistance to oxidation in the presence of air, TCEP is a stable reducing agent commonly used in mAb half-body formation.

A mAb half-body was generated through protein reduction using TCEP and subsequently identified by gel electrophoresis for use in this study. The superior resolution obtained between a monoclonal antibody monomer and half-body species using a TSKgel SuperSW mAb HR column is demonstrated in this application note.

Experimental Conditions

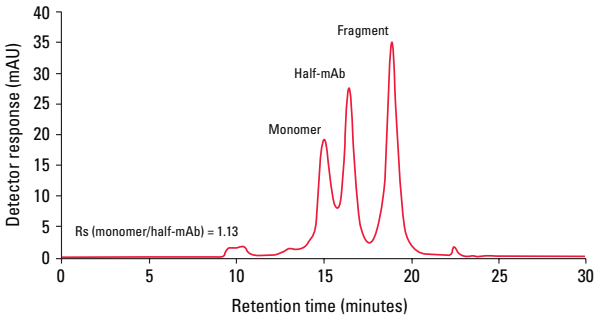
Column:	TSKgel SuperSW mAb HR, 4 µm, 7.8 mm ID × 10 cm
Mobile phase:	0.1 mol/L phosphate/0.1 mol/L sulfate buffer + 0.05% NaN_3
Flow rate:	0.5 mL/min
Detection:	UV @ 280 nm
Temperature:	25 ºC
Injection vol.:	10 µL
Sample:	human IgG (4.6 g/L) – Sigma

Results and Discussion

The complex and diverse nature of mAb structures make the reproduction of published methods difficult when using unique mAb samples. For this reason, multiple mAb reduction protocols were investigated for this study, all using TCEP Bond-Breaker® (Thermo Scientific). The use of 150 mmol/L TCEP with human IgG (4.6 g/L) incubated for 20 hours at 37 °C yielded the highest concentration of mAb half-body without excessive reduction of the protein into its low molar mass fragments. Predictably, the molar mass of the mAb half-body was approximately 70 kDa, or half that of the intact mAb.

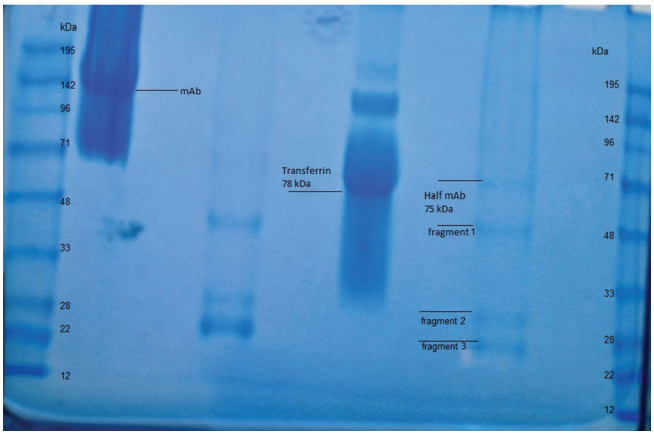
Figure 1 illustrates the separation of human IgG monomer, half-body and fragment (1/3 mAb) formed using the TCEP reduction method discussed above using a TSKgel SuperSW mAb HR column. High resolution (Rs = 1.13) of the IgG monomer and half-body species was achieved.

Figure 1. Separation of Human IgG Monomer, Half-body, and Fragments using a TSKgel SuperSW mAb HR column



SDS-PAGE was used to confirm the identity of the mAb monomer, half-body and fragment collected from the SEC separation on the TSKgel SuperSW mAb HR column. Fractions of each protein species were collected during the SEC separation and precipitated using acetone. The acetone was then removed and the protein precipitates were reconstituted in 100 µL of SDS-PAGE running buffer. The monoclonal antibody, half mAb and the fragment are clearly identified with the SDS-PAGE molar mass marker as well as transferrin (78 kDa) *(Figure 2)*. This clearly shows that the half mAb could be generated using the TCEP reduction method and separated using the TSKgel SuperSW mAb HR column.

Figure 2. SDS-PAGE Gel of Human IgG Monomer, Half-body and Fragments Separated using a TSKgel SuperSW mAb HR column.



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Conclusions

After investigation of multiple mAb reduction methods, it was determined that 150 mmol/L TCEP with human IgG incubated for 20 hours at 37 °C yielded a high concentration of IgG half-body. Separating the reduction products (IgG monomer, half-body and fragment) on the TSKgel SuperSW mAb HR column yielded high resolution (Rs of 1.13).

The TSKgel SuperSW mAb HR is able to achieve high resolution between the mAb and the mAb half-body due to its unique pore-controlled technology optimized for mAb analysis, as well as its smaller 4 µm particle size. Gel electrophoresis confirmed the identity of the reduction products separated using the TSKgel SuperSW mAb HR column. This study shows an excellent method for the separation of half-mAb or mAb half-body using the TSKgel SuperSW mAb HR column.

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