# QC Biochemistry: Monoclonal Antibody Aggregate Testing by HPLC SEC Analysis

SOP: Monoclonal Antibody Aggregate Testing by HPLC Analysis Document No: QCB 12
This SOP can be downloaded from the NBC2 website at: http://biomanufacturing.org/curriculum-resources/program-units/quality-control-biochemistry

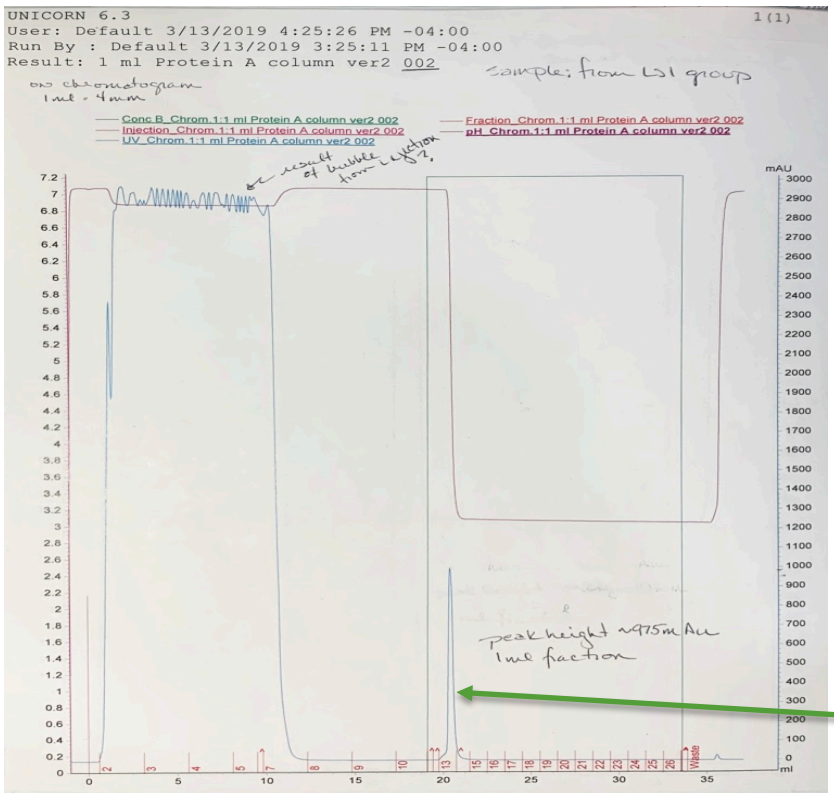
**Purpose:**
HPLC Size Exclusion Chromatography, SEC, is an analytical assay used to test the purified monoclonal antibody product for the presence of aggregates that may have resulted from growth or purification conditions. Screening for product contaminants such as aggregates is an important Quality Control requirement for both finished product and stability testing since aggregates can affect efficacy and cause an immune response in the patient.

**Materials to be analyzed:**

- Standard solution prepared using AdvanceBio SEC 300Å Protein standard
- Sample, the purified antibody fraction collected from Protein A Chromatography
- Buffer

**Procedure:**
The protein A purified anti IL-8 mAb is analyzed using HPLC SEC to determine whether there are any aggregates or fragments from the intact monomeric antibody present.

Fig. 1 AKTA protein A chromatogram




The protein A purification of the conditioned media produced a purified sample represented by a single peak in the chromatogram.

This purification step is based on the specific interaction between protein A and the Fc region of the antibody.

Purified anti IL-8 mAb

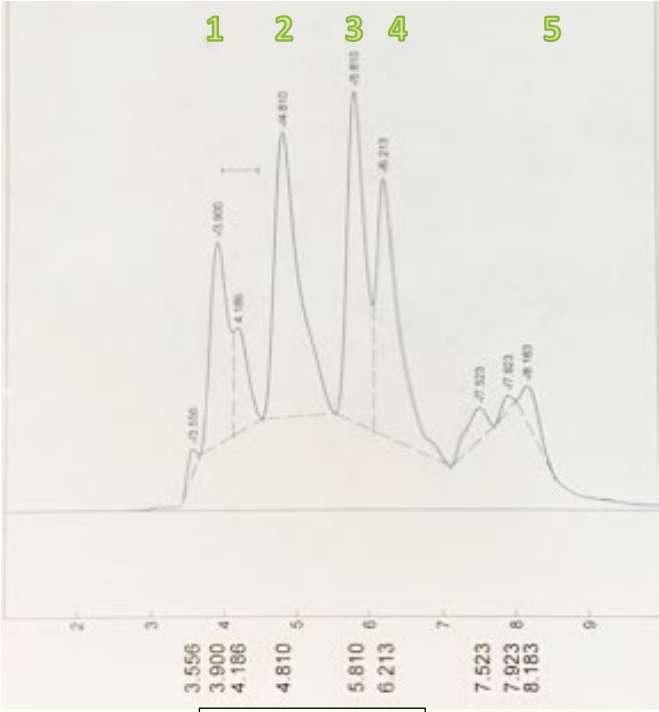
**1. Data for protein standards:**

The protein standards solution is run on the SEC column and a chromatogram generated. The retention times of each of the components along with their known molecular weights are noted.

The size range of the proteins used in the Standards solution range from 670 kDa to 1 kDa; including ɣ-globulin, which has a molecular weight of 150 kDa. Since the size of ɣ-globulin is approximately the average size of an IgG antibody, the expected retention time of an antibody IgG monomer would be very close to that observed for ɣ-globulin.

The retention times of peaks in a sample chromatogram can then be compared to the retention times of the protein standards (fig. 2) to estimate the molecular size of the peak component.

Figure 2 Chromatogram of the Standard solution




Time (minutes)

Retention times observed for the component peaks in the chromatogram of the Standards Solution.

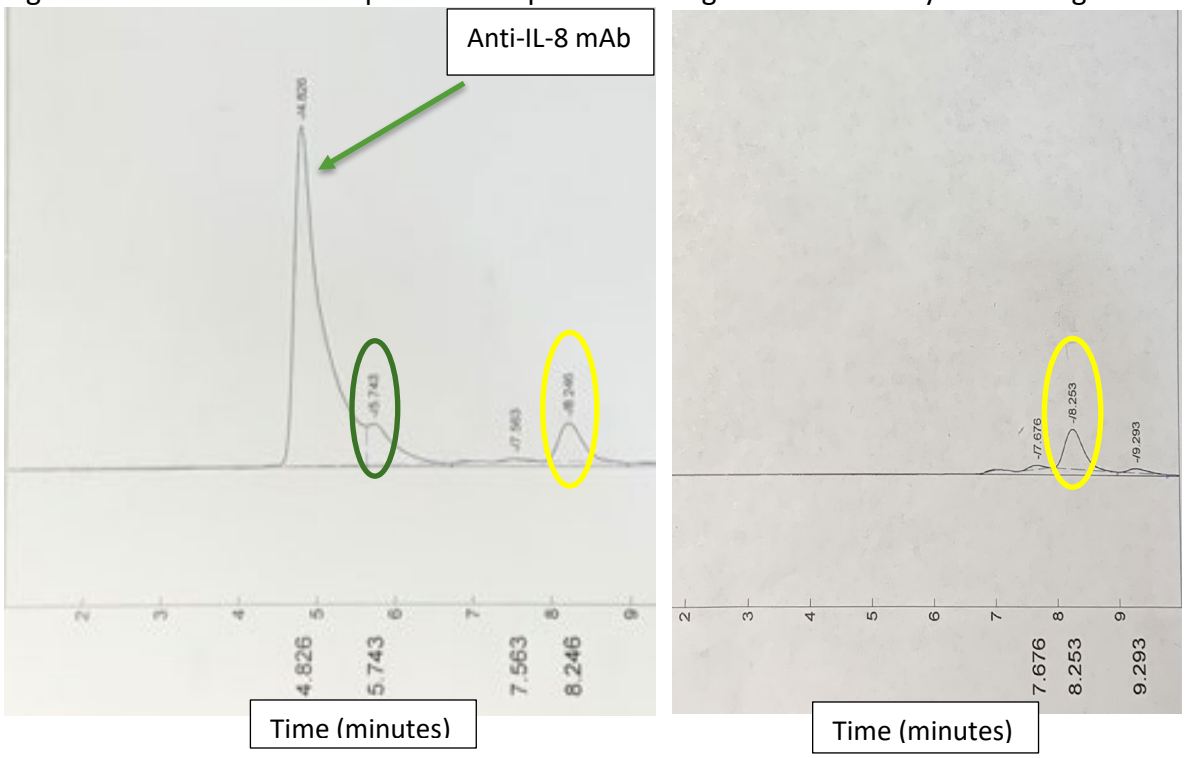
| Peak # | Standard | mwt Daltons | retention time minutes |
|---|---|---|---|
| 1 | Thyroglobulin (bovine) | 670,000 | 3.9 |
| 2 | ɣ-globulin (bovine) | 150,000 | 4.81 |
| 3 | Ovalbumin (chicken) | 45,000 | 5.81 |
| 4 | Myoglobin (equine heart) | 17,000 | 6.213 |
| 5 | Angiotensin II (human) | 1,000 | 8.183 |

Any aggregates, for example dimers, trimers or larger aggregates, would have shorter retention times and appear in the chromatogram as peaks to the left of the mAb peak. Degradation products, having a smaller size and molecular weight would appear in the chromatogram as peaks to the right of the mAb peak.

A chromatogram was generated for the buffer the sample was prepared in so that any peaks resulting from buffer components can be identified.

## 2. Anti IL-8 mAb Sample Data:

Figure 3: anti IL-8 Protein A purified sample chromatogram  Buffer only chromatogram




**Note:** Both the sample and the buffer only run depicted above were buffer exchanged to reduce the citrate concentration from the protein A elution buffer.

A sample solution for injection was prepared for both the sample and the buffer by combining 40µl the sample or buffer with 960µl of HPLC mobile phase, 100µl of this solution was injected to produce the each of the chromatograms shown. Figure 3 shows a large single peak at 4.826 minutes this retention time is very close to the retention time of the standard ɣ-globulin indicating that the molecular weight of the sample peak is close to 150,000 Daltons.
The green circled small peak on the tail of the mAb peak, with a retention time is 5.743 minutes is likely a degradation product. The closet standard molecule retention time is that of ovalbumin at 5.81 minutes. Ovalbumin has a molecular weight of 45,000 Da. This circled peak with a retention time of 5.743 minutes represents a likely degradation product of approximately 50,000 Da. Since its retention time is slightly shorter than that of ovalbumin its estimated molecular weight is slightly higher.

The yellow circled peak in both the sample and the buffer chromatogram is TRIS which absorbs at the detection wavelength of 220nm.

There are no peaks in the sample chromatogram with retention times less than that of the standard ɣ-globulin, indicating that there are no contaminants larger than the mAb, so there are no dimer or higher order aggregates in this sample.

HPLC Size Exclusion Chromatography can also be used to interpolate the molecular weight of unknown or contaminant peaks in a sample chromatogram. This is done by plotting the molecular weights of the standard solution components on the Y axis using a logarithmic scale, versus the retention times on the X axis and determining the best fit line for the linear portion of the curve. The equation of the best fit line is then used to solve for the molecular weight of an unknown peak using its measured retention time.

The accuracy of the interpolated molecular weight is dependent upon the resolution of the standard solution peaks and the range of molecular weights covered by the linear range of the standard curve.

Experiments performed and recorded by Dr. Maggie Bryans, Hetal Doshi and Robin Zuck at Montgomery County Community College. Questions regarding data can be sent to mbryans@mc3.edu.
This work was funded by NSF ATE DUE 1501631, the Northeast Biomanufacturing Center and Collaborative.