# Ion Exchange Analysis of Monoclonal Antibodies on Large Pore TSKgel BioAssist Columns

TSKgel
APPLICATION NOTE


## Abstract

TSKgel BioAssist S columns are eminently suitable for analysis of monoclonal antibodies at pH 6.0 where their stability is least affected. Similar high resolution analysis of monoclonal antibodies is possible for negatively charged antibodies on TSKgel BioAssist Q columns at pH 8.0.


## Introduction

The challenge in developing an ion exchange packing material for analyzing biopolymers is to design particles that contain large pores and have high binding capacity. Although large pores may allow full access to the available interior surface area of a particle, a simple monomeric bonded phase, as is commonly used for analyzing small molecular weight compounds, would neither result in sufficient solute retention nor in appreciable binding capacity. It has been shown[1] that so-called tentacle-type stationary phases can significantly increase the number of available binding sites, and thus binding capacity, by creating a polymer with multiple functional groups. The disadvantage of this approach is that the polymer, in turn, reduces the effective pore size. Tosoh researchers have developed crosslinked polymeric phases on large pore size particles that result in a minimal reduction of the effective pore size, while maintaining high binding capacities independent of protein molecular weight. This application note demonstrates the benefits of such materials for the analysis of monoclonal antibodies by cation and anion exchange chromatography.

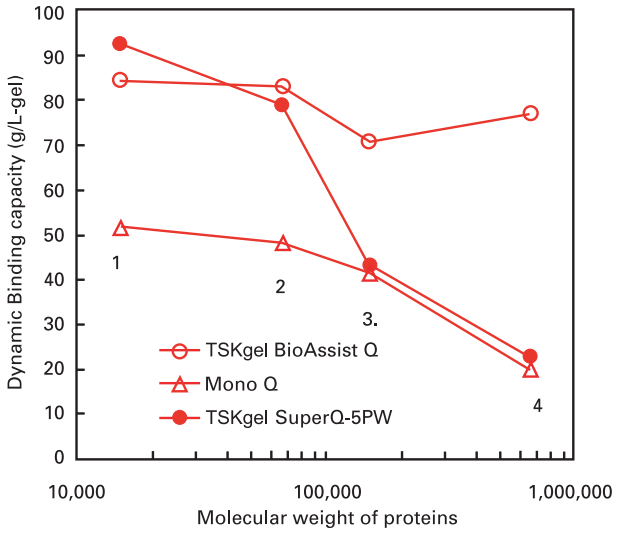
## Experimental Conditions

TSKgel BioAssist Q and S packings have a polymethyl methacrylate backbone onto which a network of polyamines (Q) or sulfopropyl (S) groups is bonded. The size of the spherical particles is either 7 micron with approximately 1300Å pores for TSKgel BioAssist S, or 10 micron with approximately 4000Å pores for TSKgel BioAssist Q. TSKgel BioAssist columns were packed in PEEK hardware of 4.6mm ID and 5cm length. Dynamic binding capacities were determined with columns of 4.6 or 5mm ID and 1cm in length. The performance of a TSKgel BioAssist Q column was compared with that of TSKgel SuperQ-5PW (Tosoh Bioscience, Montgomeryville, PA) and a 5mm ID x 5cm Mono Q column (GE Healthcare, Piscataway, NJ). Proteins and buffer components were obtained from Sigma (St. Louis, MO) or from private sources. Standard HPLC equipment was used to perform all experiments. Dynamic binding capacities were determined by continuously loading the column with a protein solution and calculating the amount of protein adsorbed from the 10% height of the breakthrough curve.

**Table 1.** Dynamic binding capacities for a monoclonal antibody on TSKgel BioAssist S and a competitive column

| Dynamic binding capacity (g/L gel) | | |
|---|---|---|
| Solution pH | TSKgel BioAssist S | Competitor A |
| 7.0 | 0 | 0 |
| 6.5 | 1.5 | 0 |
| 6.0 | 67 | 0 |
| 5.5 | 62 | 30 |

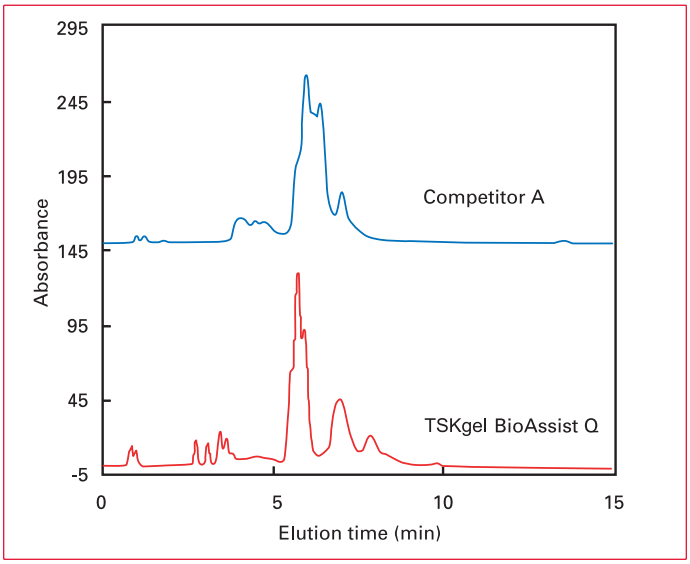
**Figure 1.** Dynamic Binding Capacity as Function of Protein Molecular Weight




## Results

The dynamic binding capacity for TSKgel BioAssist Q and two commercially available columns is shown in *Figure 1* as a function of protein molecular weight. Dynamic capacity is plotted against the molecular weight of 4 proteins varying in molecular weight from 20,000 Da. to 670,000 Da. The binding capacity on TSKgel BioAssist Q is uniformly high for all proteins, while that of Mono Q (800Å pores) and TSKgel SuperQ-5PW (1000Å pores) is distinctly lower for the larger proteins. It is evident that neither material is optimized for the analysis of monoclonal antibodies, which have a molecular weight of 150,000 Da. Antibodies are blood components and as such are most stable at pH 7.35; they become more labile at acidic pH. Their excess positive charge makes cation exchange chromatography the method of choice for their chromatographic analysis. *Table 1* shows the dynamic binding capacities for a monoclonal antibody on TSKgel BioAssist S and a competitive column. At pH 6.0 the capacity for this antibody is 67mg/mL, while the competitive column had no capacity for the antibody at this pH. *Figure 2* demonstrates that a mobile phase buffer of pH 8.0 provides high resolution analysis of mouse ascites fluid when compared to the same analysis on a competitive strong anion exchange column.

**Figure 2.** Analysis of Mouse Ascites Fluid




## Conclusions

The particle design and bonding chemistry of TSKgel BioAssist S and BioAssist Q columns make them suitable for the high efficiency analysis of monoclonal antibodies at pH 6.0 by cation exchange or at pH 8.0 by anion exchange chromatography.

## References

1. W. Müller, J. Chromatogr. 510 (1990) 133
2. E. Harris and S. Angal, "Protein Purification Methods", IRL Press, Oxford, 1989



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