

# Size Exclusion and a Well Salted Buffer



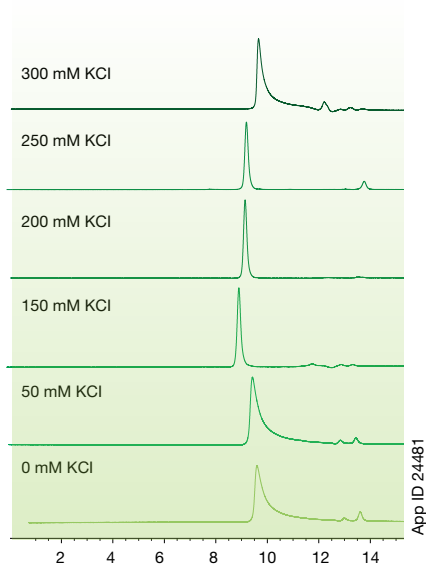
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Technical Guru - Bioseparations

### What is the best approach to optimize my mobile phase for mAb analysis by SEC?

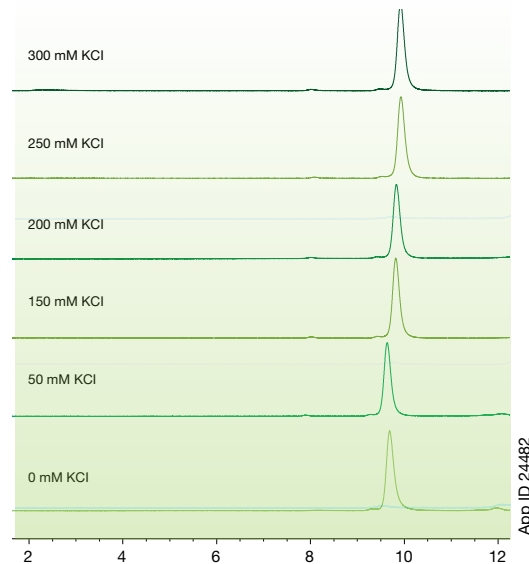
When developing a method for aggregate analysis of mAbs by SEC, it is critical to optimize mobile phase conditions to prevent non-specific secondary interactions. Below, you can see the effect of altering salt concentration in the mobile phase for two different biosimilar mAbs. The first mAb required a moderate amount of salt for acceptable peak shape. The second mAb performed well with no salt. However, increases in salt showed incremental improvements in peak shape.

Ideally, buffer and salt concentration are optimized based upon the requirements for the method or analysis. However, when there is a need for a platform method, like when needed to evaluate several different mAbs, a good starting point for method development is 50 mM monopotassium phosphate 250 mM potassium chloride, pH 6.8.

#### mAb 1, SEC Profiles



#### mAb 2, SEC Profiles



Conditions same for both samples, except where noted:

**Column:** bioZen™ 1.8 μm SEC-3  
**Dimensions:** 300 x 4.6 mm  
**Part No.:** 00H-4772-E0  
**Mobile Phase:** 50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 6.8  
KCl (as indicated)  
**Flow Rate:** 0.3 mL/min  
**Detection:** UV @ 280 nm  
**Temperature:** Ambient  
**LC System:** Agilent® 1100

# Product Ordering Information

## bioZen™ Products - Powered by Biocompatible Hardware

| bioZen Columns (mm)          | Phases      |             |             |             |             |             |             | Biocompatible Guard Cartridges* |            |
|------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------------------------|------------|
|                              | 50 x 2.1    | 100 x 2.1   | 150 x 2.1   | 250 x 2.1   | 50 x 4.6    | 150 x 4.6   | 300 x 4.6   | for 2.1 mm                      | for 4.6 mm |
| bioZen 2.6 µm Glycan         | —           | 00D-4773-AN | 00F-4773-AN | —           | —           | —           | —           | AJO-9800                        | —          |
| bioZen 1.6 µm Peptide PS-C18 | 00B-4770-AN | —           | 00F-4770-AN | —           | —           | —           | —           | AJO-9803                        | —          |
| bioZen 3 µm Peptide PS-C18   | —           | —           | —           | —           | 00B-4771-E0 | 00F-4771-E0 | —           | —                               | AJO-7606   |
| bioZen 1.7 µm Peptide XB-C18 | 00B-4774-AN | —           | 00F-4774-AN | —           | —           | —           | —           | AJO-9806                        | —          |
| bioZen 2.6 µm Peptide XB-C18 | 00B-4768-AN | —           | 00F-4768-AN | 00G-4768-AN | 00B-4768-E0 | 00F-4768-E0 | —           | AJO-9806                        | AJO-9808   |
| bioZen 3.6 µm Intact C4      | 00B-4767-AN | —           | 00F-4767-AN | —           | 00B-4767-E0 | 00F-4767-E0 | —           | AJO-9809                        | AJO-9811   |
| bioZen 3.6 µm Intact XB-C8   | 00B-4766-AN | —           | 00F-4766-AN | —           | 00B-4766-E0 | 00F-4766-E0 | —           | AJO-9812                        | AJO-9814   |
| bioZen 1.8 µm SEC-2          | —           | —           | —           | —           | —           | 00F-4769-E0 | 00H-4769-E0 | —                               | AJO-9850   |
| bioZen 1.8 µm SEC-3          | —           | —           | —           | —           | —           | 00F-4772-E0 | 00H-4772-E0 | —                               | AJO-9851   |

\*AJO-7606 requires guard holder KJO-4282. All other guard cartridges require guard holder AJO-9000.



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guarantee

If bioZen columns in this technical note do not provide at least an equivalent separation as compared to a competing column of the same phase, particle size, and dimensions, return the column with the comparative data within 45 days for a FULL REFUND.

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