# MAbPac SEC-1 Column for Monoclonal Antibody Analysis



The MAbPac<sup>™</sup> SEC-1 is a new sizeexclusion chromatography (SEC) column designed for MAb characterization, including the separation of monomers, aggregates, and fragments. The unique chemistry is stable under both denaturing and nondenaturing conditions, using high-salt, low-salt, or volatile mobile phases.

Now sold under the Thermo Scientific brand



#### **Column Chemistry**

The MAbPac SEC-1 column is based on high-purity, spherical, porous (300 Å), 5 µm silica particles covalently modified with a proprietary diol hydrophilic layer. The column is packed into nonmetallic, biocompatible, PEEK<sup>™</sup> column housing to eliminate compromised chromatography due to the presence of metal from the column hardware. As a result, the MAbPac SEC-1 column offers the following benefits:

- Minimal undesired interactions between the biomolecules and the stationary phase due to the proprietary hydrophilic-bonded layer.
- Elimination of metal contamination from the column hardware because of the nonmetallic and biocompatible PEEK housing.

- Low column bleed and compatibility with MS, ELSD and Corona<sup>®</sup> CAD<sup>®</sup> detection as a result of the stable surface bonding.
- Reproducibility and ruggedness.
- Superior performance for the analysis of MAbs, even using low-salt concentrations.

#### Analysis of MAb and Aggregates

The biopharmaceutical industry has continued its focus on the development of biotherapeutic MAbs drugs. MAbs produced from mammalian cell culture may contain significant amounts of dimers and higher-order aggregates, the formation of which originates from elevated temperature, shear strain, surface adsorption, and high protein concentration.



Passion. Power. Productivity.

Studies show that these aggregates present in drug products may cause severe immunogenic and anaphylactic reactions. Thus, biopharmaceutical manufacturers are required to develop analytical methods to monitor size heterogeneity and control levels of dimer and higher-order aggregates. SEC is a well-accepted technique for the detection and accurate quantification of protein aggregates in biological drug products. The MAbPac SEC-1 column is specially designed for quantiative separation of MAb and aggregates, providing excellent resolution between the MAb monomer and its aggregates (see chromatogram, page 1).

#### **Analysis of MAb Fragments**

MAb fragments offer various advantages over the intact protein for imaging and therapeutic applications because of their rapid pharmacokinetics and reduced nonspecific binding often associated with the glycosylated Fc portion of the immunoglobulin.<sup>1</sup> The uptake of antibody fragments by a tumor may be lower than that achieved by the intact MAb.<sup>2</sup>

The enzymes pepsin and papain have been used to digest MAbs to bivalent F(ab'), molecules that contain two antigen binding sites with a molecular mass of approximately 100 kDa,3 or the univalent Fab (one antigen-binding site) and Fc molecules, each with a molecular mass of approximately 50 kDa.<sup>4</sup> Papain is a nonspecific sulfhydryl protease that hydrolyzes most peptide bonds. Studies show that papain can enzymatically cleave MAb into two Fab and two Fc fragments in the presence of cysteine, and into one F(ab'), and one Fc fragments in the absence of cysteine.5,6 The MAbPac SEC-1 column can be used to monitor the MAb fragmentation process.



Figure 1. Analysis of MAb Fab and Fc fragments after papain digestion.

As shown in Figure 1, both  $F(ab')_2$  and Fc fragments are well resolved from the parent MAb peak and distinguished from each other in a cysteine-free, papain-digested MAb sample. It is important to note that MAbs, Fab, and Fc fragments are best separated by cation exchange chromatography using the ProPac<sup>®</sup> WCX column.<sup>7,8</sup>

### Inert Surface and Low Column Bleed

SEC is frequently used because of its simple operating principle and certain features not found in other separation techniques. The main advantage of SEC is that there are no (or only very weak) interactions between the stationary phase and the analtyes (e.g., proteins), which enables high recovery and preservation of biological activity. Moreover, because the separation is not dependent on any adsorptive property of the molecule, SEC provides a method for separating dimers, trimers, and multimers that is not easily distinguished by other chromatographic means.

Inertness (or minimal interaction with proteins) is one of the most desirable characteristics for SEC columns. The presence of any other retention mechanisms (i.e., hydrophobic interaction or ion exchange) will result in undesirable interactions and will adversely affect separation and recovery. Very often, the existence of metal or metal complexes can interact with certain proteins, and thus interfere with the analysis. Stainless steel column hardware is not fully compatible with some biological molecules, including proteins and MAbs. High salt mitigates the problem with metals and reduces the undesirable charge interaction between protein and particle surface

The MAbPac SEC-1 column utilizes a diol hydrophilic layer prepared by a proprietary process which results in an extremely low level of undesirable interaction sites. Combined with the use of the nonmetal and biocompatible PEEK column housing, it is ideal for separating MAbs (including monomer, aggregates, and fragments), by providing excellent peak shapes and efficiencies under both highand low-salt conditions. As shown in Figures 2 and 3, in high-salt conditions (0.3 M NaCl in 50 mM phosphate buffer, upper panel), both the MAbPac SEC-1 column and the competitor's column show good peak shape and peak efficiency. However, when used in lower salt concentration (0.15 M NaCl in 10 mM phosphate buffer, lower panel), the MAbPac SEC-1 column exhibits much higher efficiency and better peak shape for MAb, compared to the competitor's column.

## Compatibility with Volatile Buffer for MS, CAD, and ELSD Applications

With the growing popularity of LC–MS for characterization of biological molecules (e.g., proteins and MAbs), a column with low bleed is required. The proprietary bonding chemistry of the MAbPac SEC-1 column produces a hydrolytically stable hydrophilic bonded layer and extremely low column bleed, making it fully compatible with MS, Corona CAD, or ELSD detection.



Figure 2. MAb analysis using the MAbPac SEC-1 in high-salt and low-salt conditions.



Figure 3. MAb analysis using the competitor's column in high-salt and low-salt conditions.

Figure 4 shows the analysis of a MAb in 100 mM ammonium acetate buffer, a MS-compatible mobile phase, on both MAbPac SEC-1 (PEEK) and the competitor's columns. Note that flow rate and injection volume are adjusted to the column dimension (e.g., i.d. and column volume) to assure the columns are operated under equivalent conditions. Compared to the competitor's column, the MAbPac SEC-1 column provides superior efficiency, peak shape, sensitivity, and recovery.

## Rugged Column Packing for Reliability, Reproducibility, and Long Column Life

Rugged column packing is a critical characteristic for accurate and reproducible results, as well as good column lifetime. MAbPac SEC-1 columns are packed using a carefully developed packing protocol to ensure excellent packed bed stability, column efficiency and peak asymmetry. Figure 5 and the corresponding data in Table 1 demonstrate that even after 500 cycles of operation with intermittent injections of a MAb sample, the MAbPac SEC-1 column still maintains excellent performance, providing consistent retention time, peak shape, and peak efficiency, with minimal increase in column backpressure. The area of the dimer peak was calculated and the percent of the dimer was shown as an inset relative to the main peak.



Figure 4. MAb analysis in volatile buffer—MAb Pac SEC-1 vs the competitor's column.



Figure 5. MAb analysis using the MAbPac SEC-1 demonstrating excellent ruggedness.

Table 1. Percentage of Dimer Present in the MAb Sample (From Figure 6)						
Injection #	Monomer Retention Time	Asymmetry (at 10% Height)	Efficiency (Plates)	Dimer Retention Time	Pressure (psi)	
10	7.71	1.39	7287	6.75	1017	
100	7.71	1.36	7333	6.75	1020	
160	7.71	1.37	7310	6.75	1020	
250	7.71	1.35	7321	6.75	1027	
319	7.71	1.33	7311	6.75	1023	
467	7.71	1.35	7357	6.75	1027	
521	7.71	1.34	7357	6.75	1027	

Excellent reproducibility of monomer and dimer retention times is shown for the MAb sample.

## **Faster Separations**

The MAbPac SEC-1 is available in the 15cm shorter column length for faster separations when resolution is not critical. Figure 7 shows the MAb monomer and aggregate separation in less then four minutes.

## **Calibration Curve**

The MAbPac SEC-1 column has a wide molecular operating range as shown in the molecular weight calibration curve in Figure 6.

### **Reproducible Manufacturing**

Each MAb-SEC-1 column is manufactured to strict specifications to ensure column-to-column reproducibility. Each column is individually tested and shipped with a qualification assurance report.

#### References

- Milenic, D. E., Esteban, J. M. and Colcher, D. J. Immunol Methods 120, **1989** 71-83.
- Mather, S.J., Durbin, H. and Taylor-Papadimitriou, J. J. Immunol Methods 96, 1987 255-264.
- Nisonoff, A., Markus, G. and Wissler, F. C. *Nature* 189, **1961** 293-295.
- Parham, P. Androlewicz, M. J., Brodsky, F. M., Holmes, N. J. and Ways, J. P. *J. Immunol Methods* 53, 1982 133-173.
- 5. Liddell, J. E. and Cryer, A. A practical guide to Monoclonal Antibodies, 1991, Wiley, Chichester, UK.
- Bennett, K. L., Smith, S. V., Truscott, R. J. W. and Sheil, M. M. *Anal. Biochem.* 245, **1997** 17-27
- 7. Dionex Application Note 177 www.dionex.com
- 8. Chen, S; Lau, H, Brodsky, Y. Kleemann, G. and Latypov, R. *Protein Science* 19: 1191-1204 **2010**



Figure 6. Molecular weight calibration curve for the MAb SEC-1 column.

Table 2. Calibration Curve Proteins						
Protein	Retention Time (min)	MW (kDa)				
γ-Globulin Aggregate	7.6	7000				
Thyroglobulin Dimer	8.0	1338				
Thyroglobulin	8.9	669				
γ-Globulin Dimer	10.3	300				
BSA Dimer	11.4	134				
BSA	12.6	67				
Ovalubumin	13.3	43				
Trypsin Inhibitor	14.1	22				
Myoglobin	14.4	18				
Ribonuclease A	14.7	14				
Cytochrome C	14.6	12				



Figure 7. Faster MAb size-exclusion separation using a shorter column.

#### **SPECIFICATIONS**

Column Chemistry:
Proprietary diol
<i>Particle Size:</i> 5 μm
Pore Size: 300 Å
Separation Range:
10,000–1,000,000 (for
globular proteins)
<i>Operating pH Range:</i> 2.5–7.5
<i>Operating Temperature (max.):</i> 30 °C
Typical Flow Rate:
0.2–0.3 mL/min
Maximum Flow Rate:
0.4 mL/min
Column Housing:
PEEK

#### **ORDERING INFORMATION**

In the U.S., call (800) 346-6390 or contact the Dionex Regional Office nearest you. Outside of the U.S., order through your local Dionex office or distributor. Refer to the following part numbers:

Description	Part Number
MAbPac SEC-1 Analytical Column	
MAbPac SEC-1, 5 μm, 300Å,	
Analytical Column PEEK 4.0 × 300 mm	074696
MAbPac SEC-1, 5 μm, 300Å,	
Analytical Column PEEK, 4.0 × 150 mm	075592
MAbPac SEC-1 Guard Column	
MAbPac SEC-1, 5 µm, 300Å,	
Guard Column PEEK 4.0 × 50 mm.	074697

MAbPac is a trademark and CAD, Corona, and ProPac are registered trademarks of Dionex Corporation. PEEK is a trademark of Victrex PLC.

#### Passion. Power. Productivity.

North America

#### **Dionex Corporation**

1228 Titan Way P.O. Box 3603 Sunnyvale, CA 94088-3603 (408) 737-0700

U.S./Canada (847) 295-7500 South America Brazil (55) 11 3731 5140

#### Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294 Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0 Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190 Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380 Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

#### Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735 Taiwan (886) 2 8751 6655 www.dionex.com





LPN 2572-01 PDF 03/11 © 2011 Dionex Corporation