Chromolith® WP 300 Protein A HPLC columns

General information and guidelines for care and use

All Chromolith® Widepore columns have been extensively tested and inspected to ensure highest quality. Please examine your column for any possible damage caused in transit. If damage has occurred, immediately notify your local Merck KGaA, Darmstadt, Germany or MilliporeSigma representative and the delivery carrier.

Column information

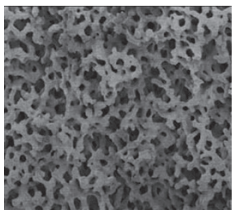
The label attached to the column indicates catalogue number, packing type, column dimensions and column number. Keep this important information with the column. If you have a problem, the column number allows us to trace the manufacturing history of your column.

Monolithic silica

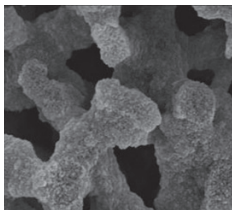
Chromolith® Widepore columns are made from a single piece of high-purity polymeric silica gel and are not packed with small silica particles. This new technology achieves a high separation performance along with a large reduction in operating pressure.

Chromolith® Widepore HPLC columns are made from highly porous monolithic rods of silica with a revolutionary bimodal pore structure providing a unique combination of macropores and mesopores. The **Macropores** allow a rapid flow of the mobile phase at low pressure.

The **Mesopores** form the fine porous structure and create the large uniform surface area on which adsorption takes place, thereby enabling high performance chromatographic separations.



a)



b)

Figure 1

Electron-microscope photographs a) Macropores; b) Mesopores

The biomodal pore structure of Chromolith® technology was tailored to the quantitation of monoclonal antibodies (mAbs) using a Protein A ligand. The unique combination of macro- and mesopores allows a fast and highly reproducible separation and quantitation of mAbs from cell culture supernatants at low column backpressures.

Specifications

Table 1

Product specifications for Chromolith® Protein A

Component	Description
Silica type	High purity (Type B)
Particle size	Monolithic
Macropore size	2 µm
Mesopore size	30 nm (300 Å)
Pore volume	1 mL
Total porosity	>80%
Surface area	~ 120 m^2/g
Immobilized ligand	Recombinant Protein A from *Staphylococcus aureus*
Pressure limit	200 bar
pH stability	1.5 - 7.5
Storage temperature	2 - 8 °C
Operating temperature	2 - 45 °C
Shipping solution	50 mM sodium phosphate + 150 mM sodium chloride pH 7.4 + 0.09% sodium azide

Connection of Chromolith® columns to HPLC systems

Chromolith® columns are cladded with a mechanically stable and chemically robust polymer (PEEK - Poly Ether Ether Ketone). The end fittings are made of the same material. Do not remove the end fittings from the column.

The end-fittings of Chromolith® columns are connected with standard 1/16" fittings to all standard HPLC, UHPLC and UPLC® systems. Short capillary tubing is recommended to minimize extra-column volumes. Install the column in the correct direction as shown on the column label.

We strongly recommend using adjustable plastic ferrules in order to avoid a possible damage to the plastic end-fittings of the Chromolith® column. The use of stainless steel ferrules is not recommended because they can damage the column end-fittings. Before connecting the column outlet to the detector, flush the column with mobile phase to remove any air.

Before connecting the column to the HPLC system, allow the column to reach room temperature.

Equilibrating the column

Chromolith® Protein A columns are shipped in 50 mM sodium phosphate and 150 mM sodium chloride pH 7.4 buffer containing 0.09% sodium azide as bacteriostatic agent. Sodium azide is a highly UV absorbing agent and will lead to a strong response when the column is connected to the detector during the equilibration process.

We recommend the removal of the shipping solution from the column with starting buffer for at least 5 column volumes (CV) and an equilibration of the column for additional 10 CV of starting buffer using a flow rate 2.0 mL/min.

Strongest interaction between Protein A and monoclonal antibodies is achieved at pH 7.0 - 7.5. In most cases, the use of buffers such as 50 mM - 200 mM sodium phosphate is recommended.

The optimum starting buffer for the use of Chromolith® Protein A contains 100 mM sodium phosphate pH 7.4. For prevention of undesired non-specific interactions, it is possible to add sodium chloride, 100 - 500 mM.

However, regardless of the used buffer system, it is necessary to use high purity reagents and salts, filtered (0.22 or 0.45 µm) prior to use.

Sample preparation

For samples with large quantities of contaminants, we recommend to apply one or more sample preparation methods prior to separation (e.g. solid phase extraction, filtration, centrifugation, etc.). Make sure that your samples and the mobile phases are clean and particulate free by using HPLC grade solvents and reagents.

We recommend the sample should be in the equilibration/starting buffer to ensure efficient binding between analyte and Chromolith® Protein A column. Additionally, you will increase your column lifetime by filtering your samples (0.22 or 0.45 µm) before injection.

The minimum and maximum load is determined by the linearity of the standard curve and the sensitivity of the used system. Generally, a Chromolith® Protein A column shows a linearity in a range of 0.25 µg - 200 µg injected antibody which is corresponding to antibody concentrations of 0.25 g/L - 10 g/L. However, we recommend the performance of your own standard curve at your system using your own conditions.

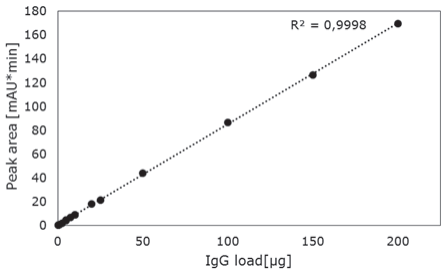


Figure 2

Standard curve of Chromolith® Protein A column for monoclonal antibody
Data point are mean values of triplicate sample injection (RSD < 2%).
Methods conditions are listed in Table 2.

Table 2
Method conditions used for figure 2, figure 3 and figure 4

Column	Chromolith® WP Protein A		
System	Dionex UltiMate 3400RS		
Flow	2.0 mL/min		
Injections	20 µL monoclonal IgG, 0.25 g/L - 10 g/L		
UV detection	280 nm		
Equilibration buffer A	100 mM sodium phosphate pH7.4		
Elution buffer B	100 mM sodium phosphate pH 2.5		
Method time table	Time [min]	Gradient [% B]	
	0.00	0	Equilibration/ Wash
	0.25	0	
	0.26	100	Elution
	1.00	100	
	1.05	0	Reequilibration
	2.50	0	

Elution

Due to the highly specific and strong interaction between immobilized protein A and Fc region of immunoglobulins, a reduction of buffer pH is necessary for the elution of the bound antibody. Most antibodies are eluted at pH 2 - 3. The optimum elution buffer for Chromolith® Protein A is a 100 mM sodium phosphate buffer pH 2.5. Use of other buffer systems is also possible, such as sodium acetate, glycine or hydrochloric acid. However, best elution conditions for the analyzed antibody should be determined empirically.

Regardless of the buffer system, it is necessary to use high purity reagents and salts, and filter those (0.22 or 0.45 µm) prior to use.

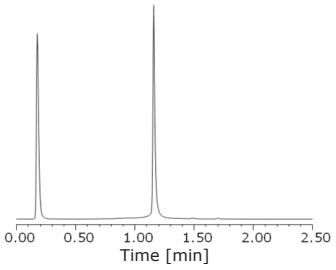


Figure 3
Separation of IgG from harvest cell culture supernatant
Method conditions are listed in Table 2.

Flow rate
Due to excellent mass transfer properties of the monolithic skeleton, high-efficiency separation is possible even at high flow rate. The separation of IgG from harvest cell culture supernatant demonstrates the extreme time savings and high separation efficiency made possible with Chromolith® Protein A columns. The IgG was well separated with excellent peak symmetry. At 5 mL/min with a 4.6 mm internal diameter column, the total analysis time is less than 1 minute and the net column backpressure is only 21 bar.

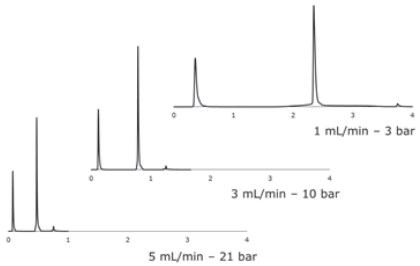


Figure 4
Separation of IgG from harvest cell culture supernatant at different flow rates
Method conditions are listed in Table 2. Elution gradient was adjusted for every flow rate, respectively.

Table 3
Comparison of column performance at different flow rates

Flow rate	Unbound area	IgG area	Pressure [bar]
1.0 mL/min	39%	61%	3
2.0 mL/min	39%	61%	6
3.0 mL/min	39%	61%	10
4.0 mL/min	39%	61%	13
5.0 mL/min	39%	61%	21

Reproducibility
For mAb titer determination, it is necessary to use a column offering a high reproducibility. Chromolith® Protein A offers highly reproducible elution of bound antibodies with constant retention time and low change in peak area. The relative standard deviation for retention time of the eluted antibody is <0.1% and for peak area <0.5% for a 50-fold injection.

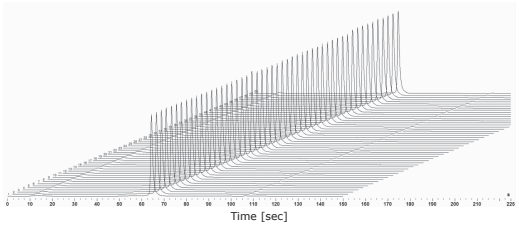


Figure 5
50-fold injection of IgG
Method conditions are listed in Table 2.

Cleaning and regeneration

The robustness of monolithic columns is superior to particulate columns due to consisting of one piece of silica. The silica skeleton provide a higher stability and the specially designed bimodal pore system reduces the risk of column blockage.

Column lifetime is highly dependent on the sample and conditions, and cannot be generalized. Lifetimes of 2000 - 3000 injections have been reported, internal tests had shown that the Protein A modification is stable for more than 50.000 CV and 10.000 pH shifts. However, with continuous use of the column, it is necessary to monitor the column backpressure and to check the column performance with a control sample periodically. Furthermore, an assessment of column linearity should be made after cleaning and regeneration.

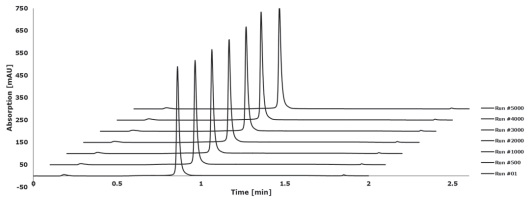


Figure 6
Stability test with 5000 runs consisting of two pH shifts
Method conditions are listed in Table 2.

Reverse the flow periodically to prevent particles and non-eluting sample components from accumulating on the column. When reversing the flow, flush the column before connecting it to the detector. It is also recommended to use for such purpose 150 mM phosphoric acid or equilibration buffer with 500 mM sodium chloride and wash the column with at least 10 CV.

Another possibility to prolong the column lifetime is the use of a guard column to protect the column top and to minimize column fouling.

Storage

Generally, it is advantageous to "wash" the column after use and before storage to remove trace of samples and buffers from the column as described in the previous chapter. Additionally, it is necessary to use the endcaps every time the column is not in use to prevent drying of the column. Drying will damage the column and decrease the column performance and lifetime.

For short-term storage, it is possible to use the initial buffer compositions as long as the column endcaps are in place.

When storing the column for several days or longer, it is important to use a bacteriostatic agent to prevent column fouling. Optimum storage conditions includes the use of neutral pH solution with a bacteriostatic agent such as the starting buffer with 0.1% sodium azide with storage in the refrigerator at 2 - 8 °C.

Ordering information for Chromolith® WP 300 products

Column dimension							
Length (mm)		ID (mm)	RP-18	RP-8	RP-4	Protein A	Epoxy
Chromolith® WP 300 HPLC Column [1 unit]							
25	x	4.6				1.52258.0001	1.52252.0001
25	x	2				1.52358.0001	1.52352.0001
50	x	4.6	1.52271.0001	1.52266.0001	1.52261.0001		1.52251.0001
50	x	2	1.52371.0001		1.52361.0001		1.52351.0001
100	x	4.6	1.52270.0001	1.52265.0001	1.52260.0001		1.52250.0001
100	x	2	1.52370.0001		1.52360.0001		1.52350.0001
Chromolith® Guard cartridges [3 units]							
5	x	4.6	1.52273.0001	1.52268.0001	1.52263.0001		1.52254.0001
5	x	2	1.52372.0001		1.52362.0001		1.52353.0001
10	x	4.6	1.52272.0001	1.52267.0001	1.52262.0001		1.52253.0001
Chromolith® Guard cartidge Holder							
5	x	4.6	1.52032.0001				
10	x	4.6	1.52033.0001				
Chromolith® Guard cartridge Holder							
for dimension			**Material**	**Item No.**			
5	x	2	Bioinert	1.52355.0001			
5	x	4.6	Bioinert	1.52255.0001			
10	x	4.6	Bioinert	1.52256.0001			

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Made in Germany

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