



Rapid and Robust Antibody Quantitation

Chromolith[®] WP 300 Protein A HPLC columns

Affinity chromatography is a selective technique which takes advantage of specific molecular interactions, for example antigen and antibody. The Chromolith® WP 300 Protein A HPLC column is designed to monitor monoclonal antibody titer and yield determination from cell-culture supernatants. This analytical scale procedure helps to optimize the titer of monoclonal antibody for the optimal time for harvest of the monoclonal antibody products.

Demonstrated Linearity Across Injection Volumes

40

20

0

0

10

20

Area

Columns:	Chromolith [®] WP 300 Protein A, 2.5 cm x 2.0 mm I.D.			
Mobile phase:	[A] 100 mM Disodium hydrogen phosphate, pH 7.4; [B] 100 mM Disodium hydrogen phosphate, pH 2.5			
Gradient:	Time	%A	%B	
	0.00	100	0	
	0.50	100	0	
	0.55	0	100	
	2.60	0	100	
	2.65	100	0	
	5.00	100	0	
Flow rate:	0.38 mL/min			
Detection:	UV, 280 nm			
Column temperature:	25 °C			
Injection volume:	2.0 µL			
Sample:	Gammanorm, 1 mg/mL, water			
140 120- *100- 80- 	- Arrow Arrow		2.635x + 1.3669 ² = 0.9996	

Key Benefits:

- Rapid and accurate titer determination across a wide range of injection volumes
- Consistent binding efficiencies even at high flow rates
- Expanded column lifetimes







30

Volume in µL

40

50

Constant Binding Efficiency at Elevated Flow Rates

- High-speed separation at high flow rate due to excellent mass transfer properties of the monolithic skeleton
- At 1 mL/min on a 2 mm I.D. column, the total analysis time is less than 1 minute and the net column backpressure is only 24 bar
- Antibody binding is not affected by flow rate

Flow Rate	Unbound area	IgG area	Pressure	
0.19 mL/min	11%	89%	5 bar	
0.38 mL/min	11%	89%	10 bar	
0.57 mL/min	11%	89%	15 bar	
0.76 mL/min	11%	89%	19 bar	
0.95 mL/min	11%	89%	24 bar	



Ordering Information

Part Number	Description	Length (mm)	I.D. (mm)
1.52358.0001	Chromolith WP 300 Protein A Column	25	2
1.52258.0001	Chromolith WP 300 Protein A Column	25	4.6

To place an order or receive technical assistance

Order/Customer Service: SigmaAldrich.com/order Technical Service: SigmaAldrich.com/techservice Safety-related Information: SigmaAldrich.com/safetycenter MilliporeSigma 400 Summit Drive Burlington, MA 01803

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< 3.2% Peak Area Absorbance at 280 nm 3 ó 4 5 6 Retention Time (minutes) Run #3000 ---- Run #2000 - Run #1000

Expanded Column Lifetimes Chromolith® WP 300 Protein A column offers excellent

reproducibility and stability for the long-term. In the below example, more than 3000 injections (> 80,000 column volumes, 6000 pH shifts) were performed on the same column, with no change in chromatographic performance.

