



Agilent Bio-Monolith HPLC Columns

Column Specifications

Agilent Bio-Monolith HPLC columns provide high resolution and rapid separations of antibodies (IgG, IgM), plasmid DNA, viruses, phages and other macro bio-molecules. The product family offers strong cation exchange, strong and weak anion exchange and Protein A phases. Bio-Monolith HPLC Columns are compatible with HPLC and preparative LC systems, including Agilent 1100 and 1200 HPLC systems

- Polymer-based, monolith HPLC columns designed for macro bio-molecule separations
- Flow rate independent separations; no diffusion, no pores and no void volume make transport between mobile and stationary phase very rapid
- Monolith disc is 5.2 mm x 4.95 mm (100 µL column volume) with continuous channels, eliminating diffusion mass transfer
- Extremely fast separations speed up method development time and decrease costs. Locking in method parameters takes significantly less time and buffer.

Dimensions	5.2 mm x 4.95 mm
Column volume	100 µL
Maximum pressure	150 bar (15 MPa, 2200 psi)
Temperature min/max	Working: 4°C-40°C (39°F-122°F) Storage: 4°C-30°C (39°F-73°F)
Recommended pH	Working range: 2-13 Cleaning-in-place: 1-14
Materials of construction	Hardware: Stainless steel Packing: poly (glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
Color ring identifier	Bio-Monolith QA: Blue Bio-Monolith DEAE: Green Bio-Monolith SO3: Red Bio-Monolith Protein A: White
Shelf life/expiration date	Protein A: 12 months So3, QA, DEAE: 24-36 months



Agilent Bio-Monolith HPLC Column Selection Guide

Column	Description	Key Applications	Part No.
Bio-Monolith QA	The quaternary amine bonded phase (Strong Anion Exchange) is fully charged over a working pH range of 2-13, binding negatively charged bio-molecules.	<ul style="list-style-type: none"> • Adenovirus process monitoring and quality control • IgM purification monitoring and quality control • Monitoring DNA impurity removal • Monitoring endotoxin removal • HSA Purity 	5069-3635
Bio-Monolith DEAE	The diethylaminoethyl bonded phase (Weak Anion Exchange) offers increased selectivity of bio-molecules with negative charge over a working pH range of 3-9.	<ul style="list-style-type: none"> • Process monitoring and quality control of bacteriophage manufacturing and purification • Process monitoring and quality control of plasmid DNA purification 	5069-3639
Bio-Monolith So3	The sulfonyl bonded phase (Strong Cation Exchange) is fully charged over a working pH range of 2-13, binding positively charged bio-molecules.	<ul style="list-style-type: none"> • Fast and high resolution analytical separations of large molecules such as proteins, antibodies • Hemoglobin A1c fast analytics 	5069-3637
Bio-Monolith Protein A	The Protein A affinity column is designed for the analytical separation of all IgG (human and mouse), except for IgG class3.	<ul style="list-style-type: none"> • Quantitative Determination of IgG (fermentation titer calculation) 	5069-3639

Bio-Monolith DEAE Column Monitors Phage Production During Fermentation

Column: DEAE
5069-3636
2 x 4 mm

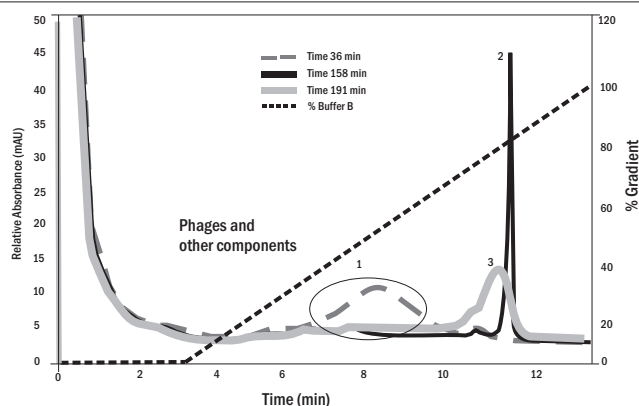
Mobile Phase: A: 125 mM Phosphate buffer, pH 7.0
B: 125 mM Phosphate buffer + 1M NaCl, pH 7.0

Flow Rate: 1 mL/min

Gradient: 100% buffer A (2.5 min)
0-100% buffer B (10 min)
100% buffer A (2 min)

Detector: UV at 280 nm

Instrument: High pressure gradient HPLC system, Agilent 1200



As phage proliferation progresses, the genomic DNA (gDNA) concentration increases as the host cells are being lysed. In the late stages of fermentation gDNA begins to degrade into fragments. These gDNA fragments cannot be easily removed by purification media so, it is critical to stop the fermentation cycle prior to the degradation of the genomic DNA. The chromatogram above represents three samples taken from the bioreactor at time 36 min, 158 min and 191 min. Peak 1 represents phage, media and host cells, peak 2 the intact gDNA and peak 3 the fragmented gDNA.

Bio-Monolith Protein A Column Rapidly Monitors IgG Fermentation Titers

Column: Protein A
5069-3636
2 x 4 mm

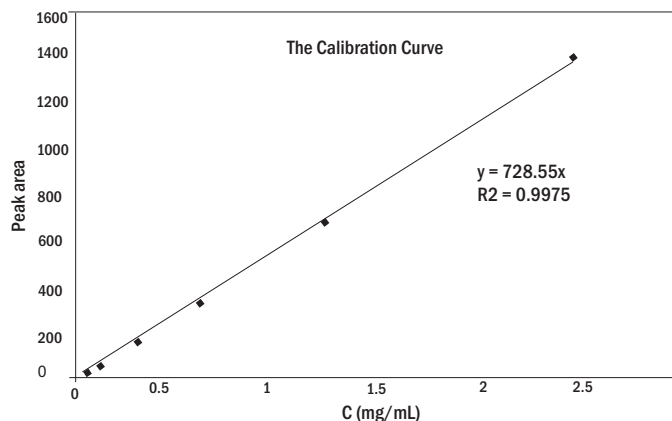
Mobile Phase: A: 1x PBS, pH 7.0
B: 0.5 M acetic acid, pH 2.6

Flow Rate: 1 mL/min

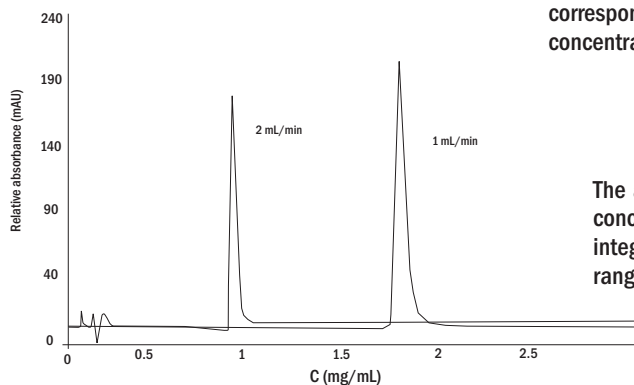
Gradient: 100% buffer A
100% buffer B
100% buffer A
(0.5 min each step)

Detector: UV at 280 nm

Instrument: High pressure gradient HPLC system, Agilent 1200



An IgG standard was measured at two flow rates, 1 mL/min and 2 mL/min, which correspond to 10 column volumes/min and 20 column volumes/min, respectively. The concentration of IgG in the sample can be reliably obtained within minutes.



The above calibration curve results from a 2-fold dilution series of human IgG concentrate. The initial IgG concentration was 2 mg/mL. IgG peak areas were integrated and plotted. The linear range of the assay easily covers the production ranges necessary to accommodate development and manufacturing cell culture

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