[PRODUCT SOLUTION]

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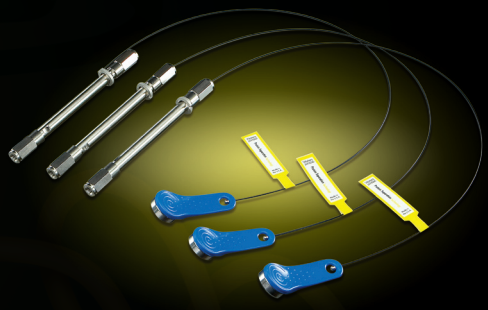
PROTEIN-PAK HI RES ION-EXCHANGE (IEX) COLUMNS FOR ACQUITY UPLC APPLICATIONS

Protein-Pak™ Hi Res Ion-Exchange (IEX) columns were developed to assist in the characterization of recombinant proteins, monoclonal antibodies, and other biological compounds. The non-porous, high compound binding capacity of these particles yields outstanding resolution of charged species in less time compared to use of many traditional porous IEX offerings. In addition, quality control testing with defined protein standards helps ensure consistent batch-to-batch performance.

- Designed for the characterization of protein charge variants and other biocompounds.
- Two cation-exchangers (Carboxymethyl and Sulfopropyl) and one anion-exchanger (Quaternary ammonium) that address selectivity needs.
- Non-porous, high-capacity stationary phases deliver fast separations that address high-through put needs.
- QC-Tested with protein standards to ensure batch-to-batch consistency.
- eCord™-enabled to help monitor column use on ACQUITY UPLC Systems.

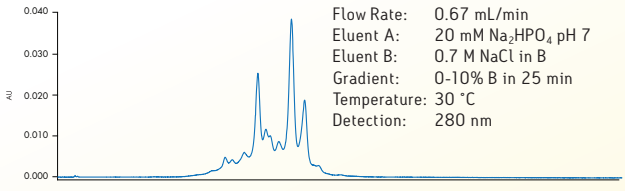


Protein-Pak™ columns

Resolved Monoclonal Antibody (mAb) Product from Variants in Less Time

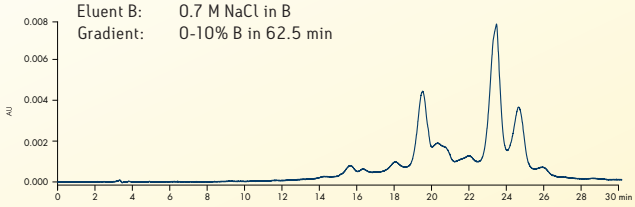
Waters Protein-Pak™ Hi Res CM, 7 µm, 4.6 x 100 mm Column

Flow Rate: 0.67 mL/min
Eluent A: 20 mM Na_2HPO_4 pH 7
Eluent B: 0.7 M NaCl in B
Gradient: 0-10% B in 25 min
Temperature: 30 °C
Detection: 280 nm



Competitor's Weak-Cation Exchanger, 4 x 250 mm Column

Flow Rate: 0.51 mL/min
Eluent A: 20 mM Na_2HPO_4 pH 7
Eluent B: 0.7 M NaCl in B
Gradient: 0-10% B in 62.5 min

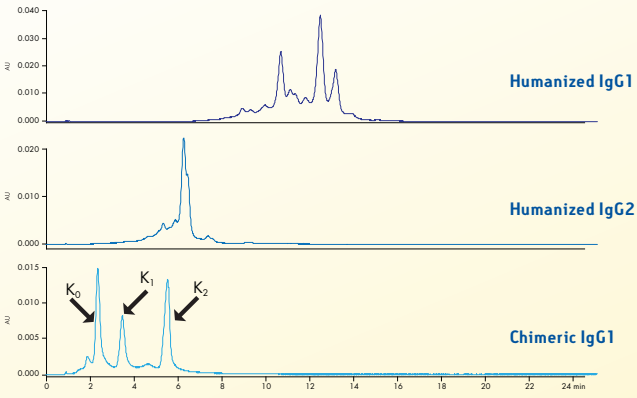


Cation-Exchange chromatography is a useful tool for the characterization and quantitation of mAb or recombinant protein variants. Use of Waters Protein-Pak Hi Res CM column on an ACQUITY UPLC System increases sample throughput while maintaining resolution between intended product and undesired variants.

Protein-Pak Hi Res CM Analysis of Three mAbs Containing Different Levels of Variants

ACQUITY UPLC® System configuration guidelines for ion-exchange are described in service notes P/N 715002147A.

Column: ProteinPak Hi Res CM, 7 µm, 4.6 x 100 mm
Flow Rate: 0.67 mL/min
Mobile Phase A: 20 mM Sodium Phosphate Buffer, pH 7.0
Mobile Phase B: 20 mM Sodium Phosphate Buffer, 0.7 M NaCl, pH 7.0
Gradient: 0–10% B in 25 min
Temperature: 30 °C
Detection: 280 nm



Sequence, production, storage, and shipping conditions influence the degree of variants contained in a biotherapeutic protein. Waters Protein-Pak Hi Res CM column can successfully resolve variations that may involve as little as a single amino acid change (K_0 = No terminal lysines, K_1 = One terminal Lysine, and K_2 = Two terminal Lysines).

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ACQUITY UPLC Technology for biotherapeutic characterization.

Application of Waters UPLC Technology for Biotherapeutic Characterization

ACQUITY UPLC allows analytical chemists to reach far beyond conventional LC separations and has proven itself to be an asset to laboratories around the world. UPLC sets new standards in resolution, sensitivity, and throughput by being the first holistically-designed system that maximizes for rapid, high-resolution analyses. It has fueled hundreds of peer-reviewed papers, helps laboratories conserve resources, and has served the needs of regulatory agencies around the globe. ACQUITY UPLC simultaneously makes your laboratory more sustainable and more efficient.

Novel IEX Particles Ideal for Biomolecule Characterizations

Protein-Pak Hi Res IEX columns contain non-porous, pH tolerant, hydrophilic particles whose surface consists of a multi-layered network or either anion (5 µm) or cation (7 µm) exchange groups. This innovative particle and bonding chemistry produces particles with greater protein loading capacities than found on many traditional monodisperse, non-porous resins. This translates into columns that can resolve complex mixtures of biomolecules in comparatively short times compared to use of alternative porous or non-porous IEX column offerings.

Description	Protein-Pak Hi Res Q	Protein-Pak Hi Res CM	Protein-Pak Hi Res SP
Ion Exchange	Strong Anion	Weak Cation	Strong Cation
Functional Group	Quaternary ammonium	Carboxymethyl	Sulfopropyl
Matrix	Hydrophilic polymer	Hydrophilic polymer	Hydrophilic polymer
Particle size (µm)	5	7	7
Pore size	Non porous	Non porous	Non porous
i.d. x L (mm)	4.6 x 100	4.6 x 100	4.6 x 100
Counter ion	Cl^-	Na^+	Na^+
pH range	3-10	3-10	3-10
Temperature (°C)	10-60	10-60	10-60
pKa	10.5	4.9	2.3
Flow Rates	0.3-0.6 mL/min	0.5-1.4 mL/min	0.5-1.4 mL/min
[1]Approx Protein Binding Capacity in mgs per column (i.e., BSA for Hi Res Q Column. Lysozyme for Hi Res CM and Hi Res SP Columns)	58	33	25

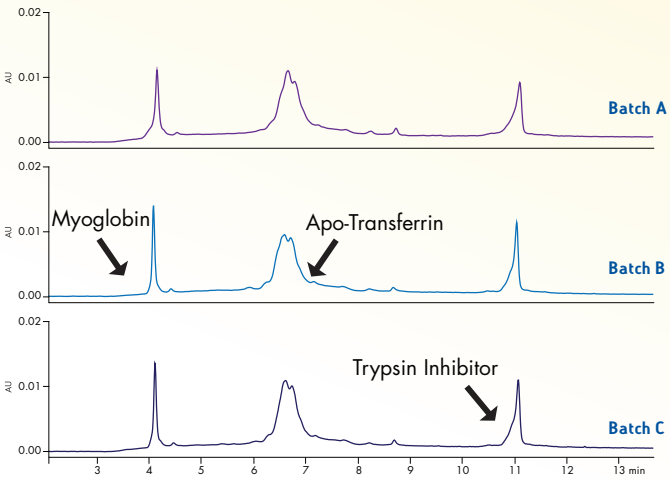
[1] For optimal resolution of complex samples, do not exceed 20% of the column's protein binding capacity.

Manufacturing Consistency for Enhanced Assurance

The ability to obtain the same high quality separations regardless of column lot is of critical importance to the successful development and commercialization of biotherapeutics. Each batch of Protein-Pak Hi Res IEX material is tested with a relevant mixture of protein standards to help ensure consistent column-to-column performance.

Column:	Protein-Pak Hi Res Q, 5 µm, 4.6 x 100 mm
Flow Rate:	0.6 mL/min
Mobile Phase A:	20 mM Tris Buffer, pH 8.5
Mobile Phase B:	20 mM Tris Buffer, pH 8.5, 0.5 M NaCl
Gradient:	0-60% B in 30 min
Temperature:	30 °C
Detection:	280 nm

Protein-Pak Hi Res IEX column Batch to Batch Reproducibility



Each batch of Protein-Pak Hi Res SP, CM, and Q column packing material is chromatography tested using a relevant protein standard mixture to help ensureconsistent and predictable performance (Protein-Pak Hi Res Q data shown).

ORDERING INFORMATION

Protein-Pak™ Hi Res CM, 7 µm, 4.6 x 100 mm	186004929
Protein-Pak™ Hi Res SP, 7 µm, 4.6 x 100 mm	186004930
Protein-Pak™ Hi Res Q, 5 µm, 4.6 x 100 mm	186004931

Note: Only when Protein-Pak Hi Res IEX columns are combined with the ACQUITY UPLC® system are the full performance benefits realized. See Waters service notes P/N 715002147A for ACQUITY UPLC system configuration guidelines for ion-exchange chromatography.

More information regarding this and additional columns for protein sample preparation and analysis can be found at **www.waters.com/Proteins**.

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