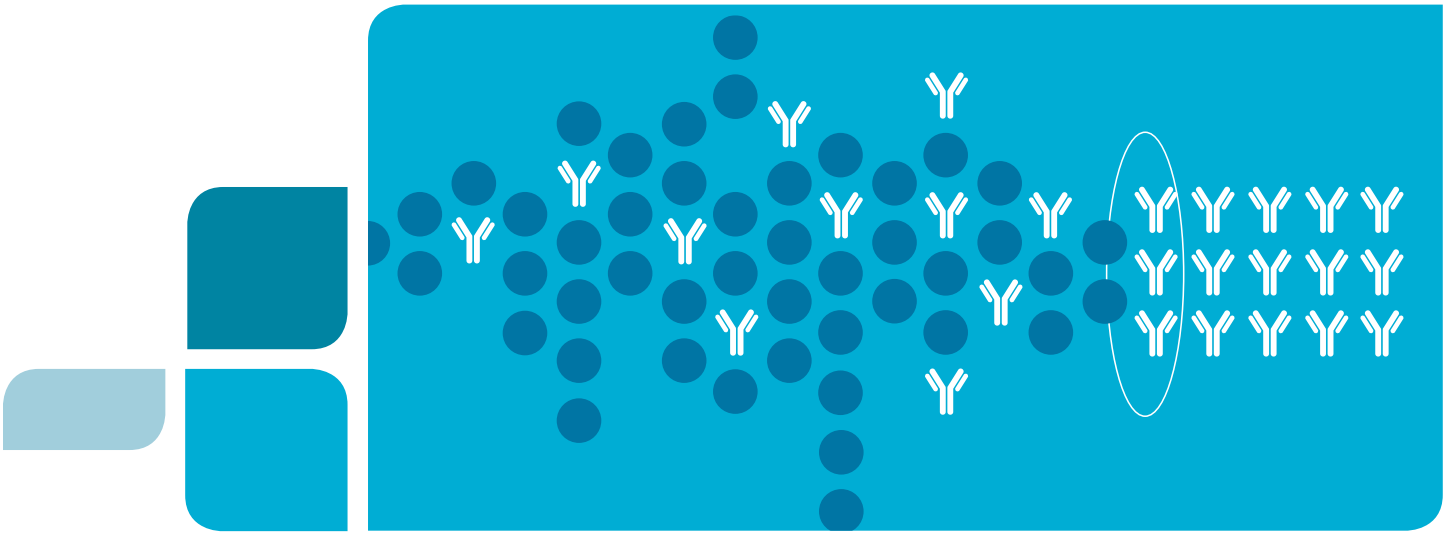

Monoclonal Antibody Purification: Intermediate Purification Resins

- Remnant impurities from capture step removed
- Closely related species resolved
- Target mAb concentration increased by decreasing impurities

Ion Exchange Resins for Monoclonal Antibody Intermediate Purification

Monoclonal antibody (mAb) purification processes typically involve a multistep workflow consisting of two or three steps for capture, intermediate, and polish purification. The resins selected for each of these steps must be compatible with the specific purification challenges that exist at that particular phase of purification.

Intermediate Purification Objectives

- Remove remnant impurities left over from the capture step
- Increase the target mAb concentration by decreasing the amount of impurities

Ideal Features for Intermediate Purification Resins

- High binding capacity to get the most efficient separation of impurities from the mAb
- Capable of separating/resolving closely related species and isoforms

Bio-Rad's Resins for mAb Intermediate Purification

- UNOsphere™ Q Anion Exchange Resin
- Nuvia™ Q Anion Exchange Resin
- Nuvia™ HR-S Cation Exchange Resin

CAPTURE	INTERMEDIATE	POLISH
UNOsphere SUPrA™	UNOsphere Q	CHT™ Ceramic Hydroxyapatite
UNOsphere SUPrA	Nuvia Q	Nuvia™ cPrime™
Nuvia™ S	Nuvia HR-S	Nuvia cPrime

BIO-RAD

UNOsphere Q Anion Exchange Resin

UNOsphere Q is a high-capacity high-throughput anion exchange resin, based on acrylamido and vinylic monomers. Its large-diameter pores and large surface area maximize binding speed, macromolecule capacity, recovery, and productivity.

Bead Properties

Property	Description
Type of ion exchanger	Strong anion
Functional group	$-N^{+}(CH_3)_3$
Particle size	120 ± 15 µm
Total ionic capacity	120 µeq/ml
Dynamic binding capacity	≥180 mg/ml at 150 cm/hr ≥125 mg/ml at 600 cm/hr 10% BT capacity determined with 2.0 mg/ml BSA in 1.1 x 10 cm column
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 1,200 cm/hr in DI water (20 x 20 cm packed bed, 1.20 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.15 –1.20
pH stability	1–14
Shipping solution	20% ethanol or 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 M NaOH
Chemical stability	
1.0 M NaOH (20°C)	Up to 10,000 hr
1.0 M HCl (20°C)	Up to 200 hr
Shelf life	5 years

BT, breakthrough; BSA, bovine serum albumin.

Performance Advantages

- **UNOsphere performance** — high dynamic binding capacity (DBC) and productivity at high flow rates
- **Superior column packing efficiency** — high efficiency at very low flow rates, which remains good at rates up to 1,200 cm/hr; uniform packing and no channeling or interaction of the sample with the support
- **Excellent base stability** — little effect on DBC even after extended storage in both 0.1 N and 1.0 N NaOH
- **Stable retention times** — virtually identical retention times for several test proteins at up to 10,000 hr of storage in 1.0 N NaOH
- **Solvent independent stability** — favorable pressure and flow properties in the presence of common chaotropic agents and at various pH values and salt concentrations
- **Optimized bead design** — to maximize capture speed of impurities from capture eluent or initial feed
- **Unparalleled quality** — for batch-to-batch reproducibility

Competitive Data

Capacity, recovery, and productivity of UNOsphere Q and two other anion exchange resins. The pressure/flow properties of UNOsphere Q (Bio-Rad Laboratories), Q Sepharose FF (GE Healthcare), and Fractogel EMD TMAE (EMD Millipore) were evaluated on a 1.1 x 20 cm (20 ml) column equilibrated with 10 mM Tris buffer, pH 8.5 (buffer A). Bovine serum albumin (BSA; 5.0 mg/ml in buffer A) was loaded until 10% breakthrough occurred. Elution was performed with buffer A containing 0.5 M NaCl. Chromatography was performed on a BioLogic DuoFlow™ System. UNOsphere Q exhibits best-in-class DBC, recovery, and productivity, in part due to its open architecture and low backpressure at high flow rates (Table 1).

Table 1. Comparison of UNOsphere Q, Q Sepharose FF, and Fractogel EMD TMAE Resin properties.

Support	Linear Velocity, cm/hr	Recovery, %	BSA Binding Capacity, g/L	Process Time, hr	Productivity, g/L/hr
UNOsphere Q	615	100.0	120.0	1.58	75.0
Q Sepharose FF	300	99.0	23.0	1.19	19.0
Fractogel EMD TMAE (M)	105	99.0	82.0	5.04	16.0

Other Resources

- Instruction manual, bulletin 4110109
- Product information sheet, bulletin 2724
- UNOsphere Q Resin technical data, bulletin 2729
- Purification of a monoclonal antibody after Protein A capture, bulletin 5735
- Effective cleaning and sanitizing of anion exchange resins, bulletin 5543

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4714	**Foresight™ UNOsphere Q Plates**, 20 µl
732-4819	**Foresight UNOsphere Q RoboColumn Unit**, 200 µl
732-4820	**Foresight UNOsphere Q RoboColumn Unit**, 600 µl
732-4732	**Foresight UNOsphere Q Column**, 1 ml
732-4752	**Foresight UNOsphere Q Column**, 5 ml
Bulk Resin	
1560101	**UNOsphere Q Support**, 25 ml
1560103	**UNOsphere Q Support**, 100 ml
156-0105	**UNOsphere Q Support**, 500 ml
156-0107	**UNOsphere Q Support**, 10 L

Nuvia Q Anion Exchange Resin

Nuvia Q Resin is an ultra-high capacity high-throughput next-generation anion exchange resin. It delivers best-in-class binding capacity at high flow rates and rapid mass transfer kinetics, providing a wide experimental design space for process developers.

Bead Properties

Property	Description
Type of ion exchanger	Strong anion
Functional group	$-N^+(CH_3)_3$
Particle size	85 ± 15 µm
Total ionic capacity	100–170 µeq/ml
Dynamic binding capacity	>170 mg/ml at 300 cm/hr 10% BT capacity determined with 5 mg/ml BSA in 20 mM Tris-HCl, pH 8.5
Recommended linear flow rate	50–600 cm/hr
Pressure vs. flow performance	Under 3 bar up to 500 cm/hr in DI water
Compression factor (settled bed volume/ packed bed volume)	1.10–1.15
pH stability	Short term: 2–14 Long term: 4–12
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.01 N NaOH
Chemical stability	
1.0 N NaOH (20°C)	Up to 1 week
0.01 N NaOH (20°C)	Up to 5 years
Shelf life	5 years

BT, breakthrough; BSA, bovine serum albumin.

Performance Advantages

- **Exceptionally high binding capacity** — over a range of pH and flow rates
- **Robust stability and performance** — stable DBC and recovery even with extended storage in 1.0 N NaOH; consistent performance due to chemical stability, ensuring batch-to-batch reproducibility
- **Excellent pressure/flow performance** — high linear flow rates without proportional increase in pressure
- **Improves productivity** — contributing to reduced capital costs, space requirements, and cycle time
- **Unsurpassed quality** — for batch-to-batch reproducibility
- **Flexible format** — usable in both affinity-based and affinity-independent workflows

Competitive Data

DBC and flow rates of Nuvia Q and two other anion exchange resins. Three 1.1 cm columns were packed to a 10.6 cm bed height with Nuvia Q, agarose Q, or polymeric Q resins. BSA (5 mg/ml in 20 mM Tris-HCl, pH 8.5) was loaded onto each column until 10% breakthrough (BT) was observed. Nuvia Q exhibits the highest DBC of BSA at high linear velocities, relative to the two other anion exchange resins (Figure 1).

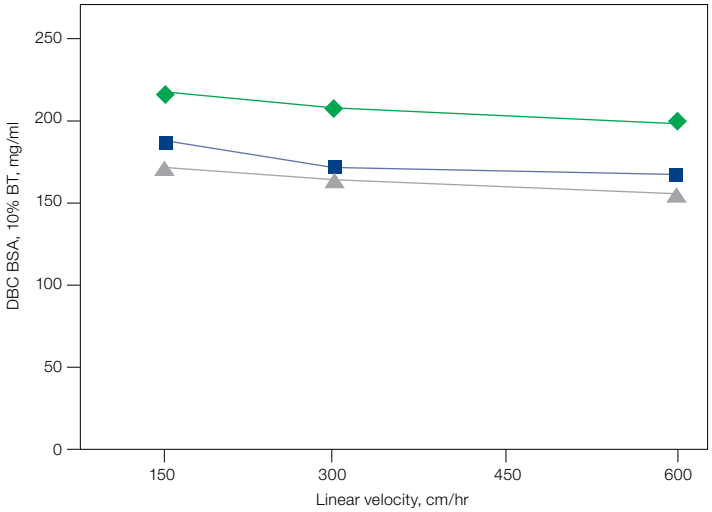

Fig. 1. Comparison of DBC vs. flow velocity of Nuvia Q Resin and two other anion exchange resins. Nuvia Q Resin (◆); agarose Q resin (■); polymeric Q resin (▲).

Other Resources

- Instruction manual, bulletin 10018215
- Product information sheet, bulletin 6129
- Automated mAb workflows: combining multidimensional (Multi-D) purifications with product analysis, bulletin 6745

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4703	**Foresight Nuvia Q Plates**, 2 x 96-well, 20 µl
732-4804	**Foresight Nuvia Q RoboColumn Unit**, 200 µl
732-4805	**Foresight Nuvia Q RoboColumn Unit**, 600 µl
732-4721	**Foresight Nuvia Q Column**, 1 x 1 ml
732-4741	**Foresight Nuvia Q Column**, 1 x 5 ml
Bulk Resin	
1560411	**Nuvia Q Media**, 25 ml
1560413	**Nuvia Q Media**, 100 ml
156-0415	**Nuvia Q Media**, 500 ml
156-0417	**Nuvia Q Media**, 10 L

Nuvia HR-S Cation Exchange Resin

Nuvia HR-S Resin is a small particle high-resolution cation exchange resin designed for intermediate and final polish purification steps where the separation of closely related biomolecules is challenging. Its exceptional selectivity and high recovery is due to optimized particle size and chemistry. It is designed to meet biomolecule purification needs at both the laboratory- and large bioprocess-scale levels.

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size	50 ± 10 µm
Total ionic capacity	100–180 µeq/ml
Dynamic binding capacity	≥70 mg/ml at 300 cm/hr 10% BT capacity determined with 5.0 mg/ml hIgG in 20 mM Na acetate, pH 5.0.
Recommended linear flow rate	50–200 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 200 cm/hr in DI water (20 x 20 cm packed bed, 1.25 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.20–1.25
pH stability	Short term: 2–14 Long term: 4–13
Shipping solution	20% ethanol
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH
Chemical stability	
1.0 N NaOH (20°C)	Up to 5 weeks (840 hr)
0.1 N NaOH (20°C)	Up to 5 years
Shelf life	5 years

BT, breakthrough; hIgG, human immunoglobulin G.

Performance Advantages

- **High binding capacity** — over a wide operating pH range
- **Excellent pressure/flow performance** — linear relationship between pressure and flow up to 200 cm/hr
- **Superior resolution** — able to separate mAb aggregates from monomers
- **Consistent and reproducible performance** — no decrease in BSA DBC and recovery when exposed to 840 hr of 1.0 N NaOH; consistent performance due to chemical stability, ensuring batch-to-batch reproducibility and repetitive use without compromising performance
- **Excellent scalability** — compatibility from bench to manufacturing steps

Competitive Data

Aggregate content and monomer recovery. Aggregate clearance and monomer recovery of 46 mg/ml of IgG/ml resin was carried out using Nuvia HR-S and an agarose-based small particle cation exchanger, Resin 1. Nuvia HR-S delivered a final aggregate content of <0.3% and a recovery of >80% while the recovery using agarose-based Resin 1 was <70% for the same final aggregate content (Figure 2). The higher capacities and better recovery translate to reduced cost of goods for downstream processes.

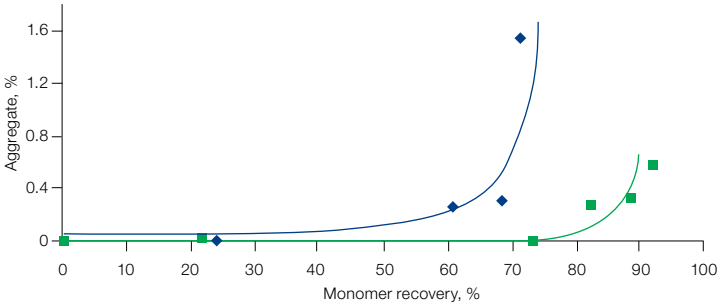

Fig. 2. Performance of Nuvia HR-S (■) vs. Resin 1 (♦).

Other Resources

- Instruction manual, bulletin 10033316
- Product information sheet, bulletin 6448
- Improving aggregate removal from a mAb feedstream using high-resolution cation exchange chromatography, bulletin 6439

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4707	**Foresight Nuvia HR-S Plate**, 20 µl
732-4831	**Foresight Nuvia HR-S RoboColumn Unit**, 200 µl
732-4832	**Foresight Nuvia HR-S RoboColumn Unit**, 600 µl
732-4723	**Foresight Nuvia HR-S Column**, 1 ml
732-4743	**Foresight Nuvia HR-S Column**, 5 ml
Bulk Resin	
1560511	**Nuvia HR-S Media**, 25 ml
1560513	**Nuvia HR-S Media**, 100 ml
156-0515	**Nuvia HR-S Media**, 500 ml
156-0517	**Nuvia HR-S Media**, 10 L

All our resins come with full regulatory support backed by Bio-Rad's global application and development team. Contact your regional Bio-Rad process chromatography specialist at process@bio-rad.com or call customer service at 1-800-4-BIORAD (1-800-424-6723) for more information.

Test drive our resins for your mAb purification.
Visit bio-rad.com/web/ResinSample to order your sample.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 1 877 89 01 177* ***Belgium*** *32 (0)3 710 53 00* ***Brazil*** *55 11 3065 7550* ***Canada*** *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *45 44 52 10 00* ***Finland*** *358 09 804 22 00* ***France*** *33 01 47 95 69 65* ***Germany*** *49 89 31 884 0* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *972 03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *31 (0)318 540 666* ***New Zealand*** *64 9 415 2280* ***Norway*** *47 23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 (0) 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *46 08 555 12700* ***Switzerland*** *41 026 674 55 05* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 662 651 8311* ***United Arab Emirates*** *971 4 8187300* ***United Kingdom*** *44 020 8328 2000*