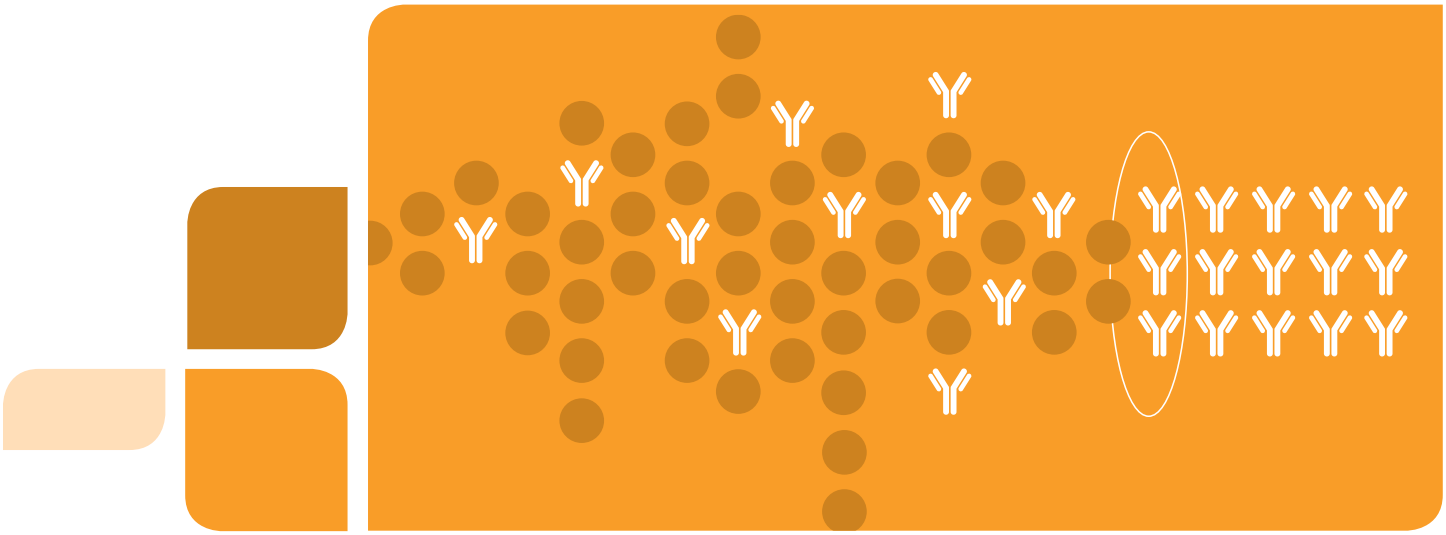

Monoclonal Antibody Purification: Capture Purification Resins

- Bulk impurities such as HCPs and DNA removed from initial feed
- Target mAb concentration increased by decreasing impurities and feed volume

Affinity and Ion Exchange Resins for Monoclonal Antibody Capture 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.

Capture Purification Objectives

- Remove the bulk of the impurities, including host cell proteins (HCPs), endotoxins, and DNA, from the initial feed
- Increase target mAb concentration by decreasing the impurities and feed volume

Ideal Features for Capture Purification Resins

- Able to withstand high flow rates in order to minimize
  - the potential of mAb inactivation/misfolding
  - target antibody's exposure to proteases and nucleases present in the cell culture feedstream
- High binding capacity to get the most efficient separation of impurities from the mAb

Bio-Rad's Resins for mAb Capture Purification

- UNOsphere SUPrA™ Affinity Resin
- Nuvia™ 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 SUPrA Affinity Resin

UNOsphere SUPrA Affinity Resin combines UNOsphere bead technology with a recombinant Protein A ligand, making it an ideal candidate for the production of mAbs. Its outstanding pressure/flow performance allows for its use in large-scale process applications.

Bead Properties

Property	Description
Ligand	Recombinant Protein A
Particle size	53–61 µm
Total ligand density	10 mg/ml
Dynamic binding capacity	30 ± 3 mg/ml 10% BT capacity determined with 1.0 mg/ml polyclonal hIgG in 1.1 x 10 cm column
Recommended linear flow rate	100–600 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 300 cm/hr in DI water (20 x 20 cm packed bed, 1.15 compression factor)
pH stability	3–11
Shipping solution	50% slurry in 20% ethanol
Regeneration	1–2 M NaCl
CIP/sanitization	6 M guanidine-HCl, 10 mM hydrochloric acid, 0.1 M sodium hydroxide, 1 M acetic acid/20% ethanol
Storage conditions	2–8°C
Chemical stability No significant change in chromatographic performance after storage at RT for 24 hr	10 mM HCl, 6 M guanidine-HCl, 0.1 M arginine (pH 2.8), 0.1 M citrate (pH 2.8), 0.1 M glycine (pH 2.8)
Shelf life	5 years (4–8°C)

BT, breakthrough; hIgG, human immunoglobulin G.

Performance Advantages

- **Excellent flow properties** — fast flow without proportional pressure spikes
- **High dynamic binding capacity (DBC) at high bed heights** — increased bed height leads to increased residence time, which leads to increased mAb binding to the resin
- **Narrow elution profile** — reduced requirement for elution buffer
- **Rapid mass transfer** — minimized aggregation or precipitation of low pH–sensitive mAbs
- **Predictable performance** — over a wide range of mAb concentrations
- **High recovery** — typically >95% target mAb recovery

Competitive Data

The low pH requirement for elution from Protein A–based resins can result in higher aggregate content and precipitation and lower monomer concentration in the recovery pool. The rapid mass transfer characteristics of UNOsphere SUPrA help overcome this challenge.

Percent recovery of two mAbs at different pH by UNOsphere SUPrA and two other Protein A resins. mAb G and mAb R were screened on three Protein A resins. Each mAb (5.5 mg) was loaded onto columns with 1 ml of each resin, run at 300 cm/hr, and eluted with 0.1 M glycine at pH 3.7. More than 80% of each mAb was recovered from UNOsphere SUPrA, whereas recovery was much less from the two other resins (Figure 1A). Since a lower pH is known to improve the performance of the other two resins, the experiments were repeated at pH 3.5. Total percent recovery for both mAb G and mAb R was more consistent with UNOsphere SUPrA than with the other resins (Figure 1B). Greater than 80% recovery can be achieved in 3–5 CV only with UNOsphere SUPrA.

A. pH 3.7

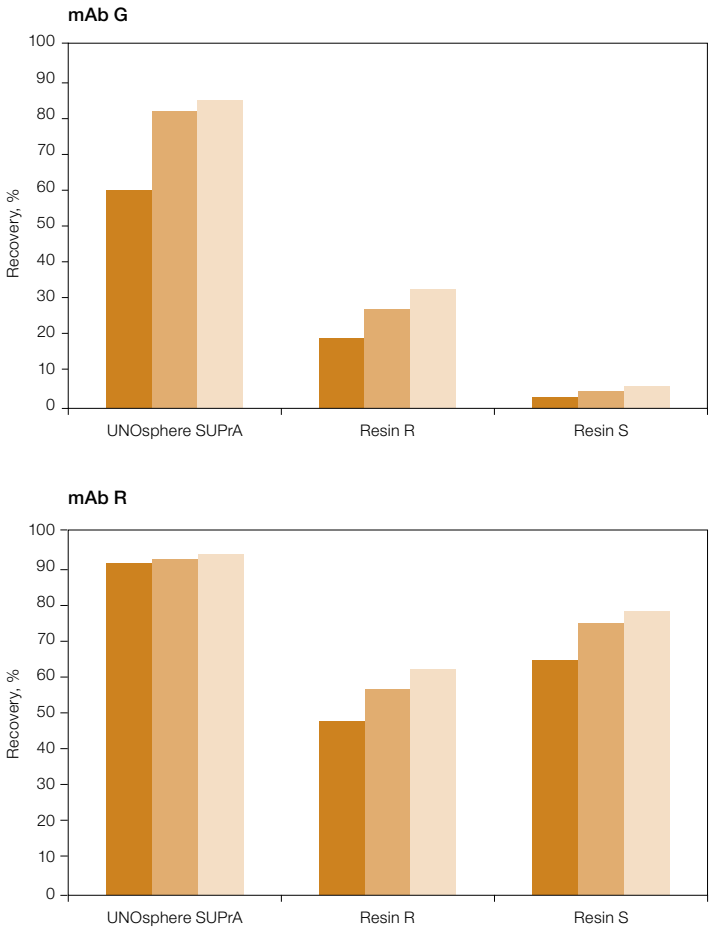

Fig. 1A. Comparison of percent recovery using UNOsphere SUPrA and two other Protein A resins at pH 3.7. Percent recovery was evaluated at pH 3.7. 3 CV (■); 5 CV (■); 7 CV (■).

B. pH 3.5

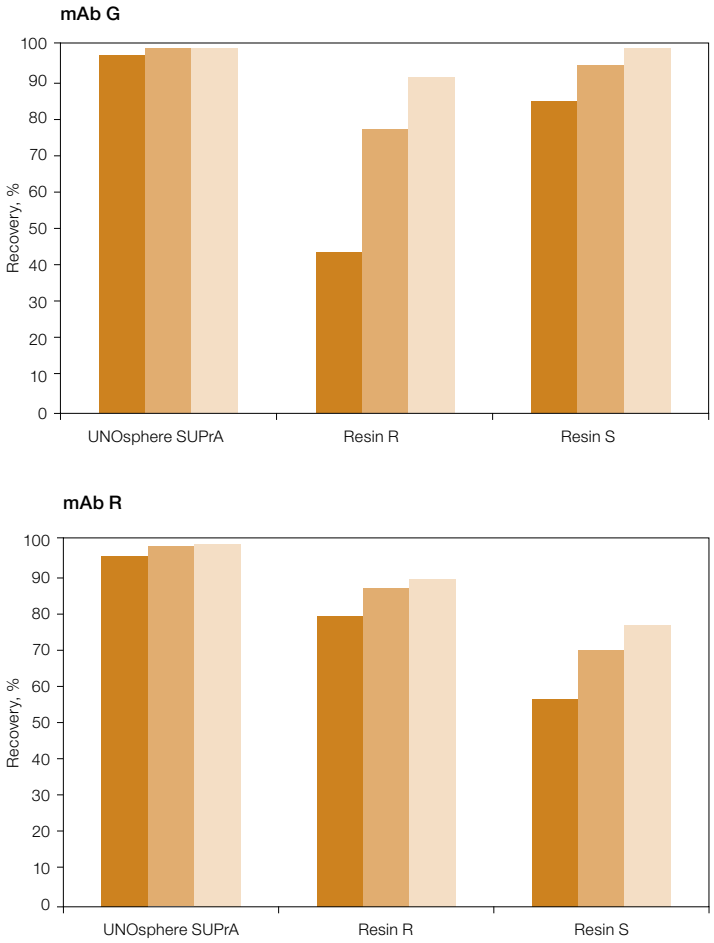

Fig. 1B. Comparison of percent recovery using UNOsphere SUPrA and two other Protein A resins at pH 3.5. Percent recovery was evaluated at pH 3.5. 3 CV (■); 5 CV (■); 7 CV (■).

Other Resources

- Instruction manual, bulletin 10014430
- Product information sheet, bulletin 5729
- Monoclonal antibody purification using UNOsphere SUPrA Resin, bulletin 5728
- Application of UNOsphere SUPrA Resin in industrial antibody purification, bulletin 6053

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4729	**Foresight™ UNOsphere SUPrA Column**, 1 ml
732-4749	**Foresight UNOsphere SUPrA Column**, 5 ml
732-4834	**Foresight UNOsphere SUPrA RoboColumn Unit**, 200 µl
732-4835	**Foresight UNOsphere SUPrA RoboColumn Unit**, 600 µl
Bulk Resin	
1560218	**UNOsphere SUPrA Affinity Chromatography Media**, 25 ml
1560219	**UNOsphere SUPrA Affinity Chromatography Media**, 100 ml
156-0220	**UNOsphere SUPrA Affinity Chromatography Media**, 500 ml

Nuvia S Cation Exchange Resin

Nuvia S Resin is built on the industry proven UNOsphere base matrix technology, designed for robust and scalable process applications. It is a next-generation resin that provides flexible design for both capture and intermediate purifications.

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size	85 ± 15 µm
Total ionic capacity	90–150 µeq/ml
Dynamic binding capacity	>110 mg/ml at 300 cm/hr 10% BT capacity determined with 4.5 mg/ml hIgG in 40 mM Na acetate + 30 mM NaCl, pH 5.0.
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2.5 bar at a flow rate of 600 cm/hr in DI water (20 x 20 cm packed bed, 1.17 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.15–1.20
pH stability	Short term: 2–14 Long term: 4–13
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 0.1 N NaOH
Chemical stability	
1.0 N NaOH (20°C)	Up to 1 week
0.1 N NaOH (20°C)	Up to 5 years
Shelf life	5 years

Performance Advantages

- **Best-in-class DBC** — superior DBC over a broad range of pH, conductivity, and flow rates due to unique design
- **Replacement of affinity capture in a three-step process** — efficient HCP and DNA clearance to effectively replace an affinity-based capture in a three step process
- **Base stability and consistent performance** — no decrease in DBC or recovery when exposed to 840 hr of 1.0 M NaOH with typically used cleaning cycles; delivers value and process stability by maintaining peak performance over an extended life
- **Economical** — in terms of cost, buffer consumption, and space requirements
- **Superior productivity** — with decreased cycle time

Competitive Data

DBC at high flow rates on Nuvia S and other commercially available CEX resins. Binding of human immunoglobulin G (hIgG) was tested on Nuvia S and three other commercially available CEX resins (Figure 2). Nuvia S exhibited the most efficient binding of hIgG in the linear velocity range 150–600 cm/hr, significantly outperforming the other CEX resins. The binding capacity of hIgG on Nuvia S was found to be 114 mg/ml even at a high flow rate of 600 cm/hr. When tested against six other commercial CEX resins, Nuvia S showed the highest DBC under similar conditions (Figure 3). Such performance allows Nuvia S to deliver the speed and throughput needed in the process manufacturing of mAbs.

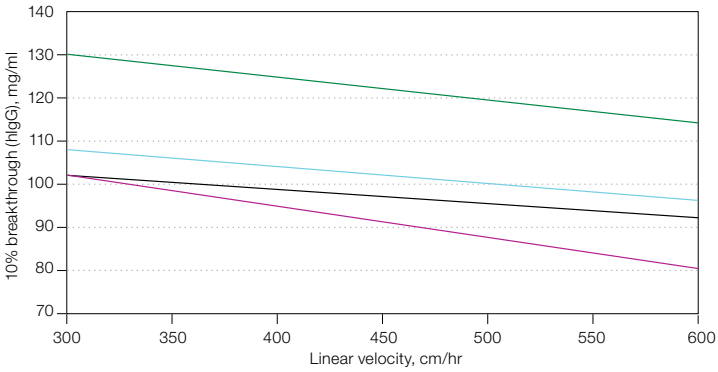

Fig. 2. Binding of hIgG by Nuvia S Resin and three other resins at various linear velocities. Column size, 1.1 x 9.5 ± 0.3 cm. Nuvia S (—); CEX 1 (—); CEX 2 (—); CEX 3 (—).

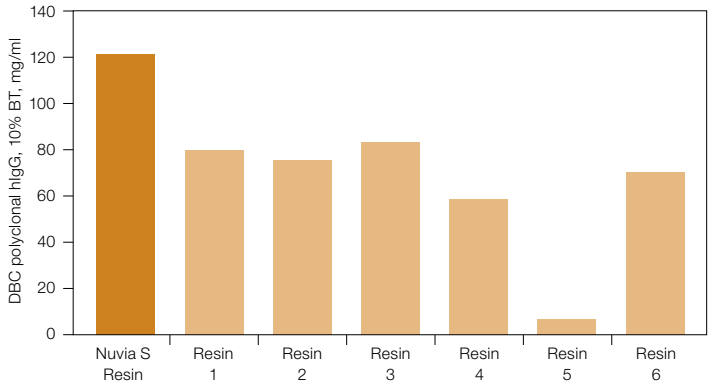

Fig. 3. Comparison of Nuvia S and other commercially available CEX resins. Column size, 0.7 x 5.5 cm. Sample was loaded onto the column, in 40 mM sodium acetate, pH 5.0 and 30 mM sodium chloride, washed, and eluted with 40 mM sodium acetate, pH 5.0 and 1 M sodium chloride. BT, breakthrough.

Other Resources

- Instruction manual, bulletin 10018215
- Product information sheet, bulletin 5987
- High-capacity CEX resin for the process purification of monoclonal antibodies, bulletin 5984

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4701	**Foresight Nuvia S Plates**, 2 x 96-well, 20 µl
732-4801	**Foresight Nuvia S RoboColumn Unit**, 200 µl
732-4802	**Foresight Nuvia S RoboColumn Unit**, 600 µl
732-4720	**Foresight Nuvia S Column**, 1 x 1 ml
732-4740	**Foresight Nuvia S Column**, 1 x 5 ml
Bulk Resin	
1560311	**Nuvia S Media**, 25 ml
1560313	**Nuvia S Media**, 100 ml
156-0315	**Nuvia S Media**, 500 ml
156-0317	**Nuvia S Media**, 10 L

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