Chromatography

Laboratory-Scale Protein Purification

Column Selection Guide

Visit bio-rad.com/Columns for more information.

BIO-RAD

Protein Purification Resins and Columns

Bio-Rad™ has a comprehensive chromatography column portfolio for high-quality protein purification. Columns are available for preparative affinity, ion exchange (IEX), size exclusion, mixed-mode, and hydrophobic interaction chromatography (HIC) for the most common and important laboratory-scale techniques. In addition, high performance liquid chromatography (HPLC) columns for small molecule analysis are included in the Bio-Rad lab-scale column collection.

Affinity

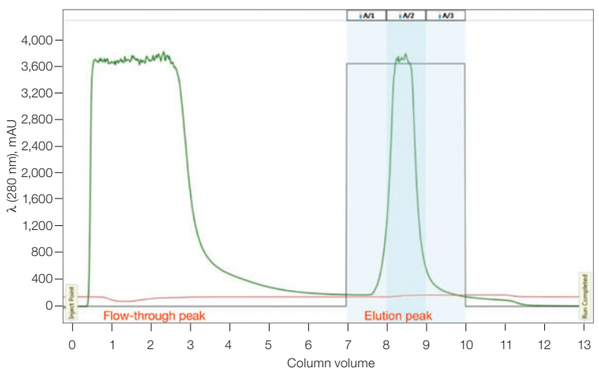
Affinity chromatography separates proteins on the basis of the attraction of a protein moiety to a ligand bound to the chromatography resin. This technique is performed by either utilizing attraction of native protein moieties to a chromatography resin or engineering a tag into the protein to interact with a specific type of resin. The separation is both selective and reversible, and the binding mechanism can be compared to a lock and key connection. Elution can be performed by using a competitive ligand, by changing the solution's ionic strength or pH, or through the use of a chaotropic agent. Affinity chromatography is typically the first step in a purification protocol, allowing high enrichment of a protein of interest.

Isolation of antibodies and other blood proteins through native attraction using affinity chromatography can be performed using prepacked columns with UNOsphere, Affi-Prep™, or Affi-Gel™ Resins.

- UNOsphere SUPrA Resin, based on recombinant Protein A, delivers a high binding capacity for antibodies
- Affi-Prep Protein A Resin yields highly purified immunoglobulin G (IgG) antibodies, selectively removes IgG prior to analysis of other immunoglobulin classes, or adsorbs immune complexes for antigen purification
- Affi-Gel Blue, DEAE Affi-Gel Blue, and CM Affi-Gel Blue Resins provide single-step isolation or removal of albumins and other blood proteins, such as IgG antibodies

Purification of tagged proteins is made easy with the Nuvia and Profinity product families, which include EconoFit Nuvia IMAC, EconoFit Profinity GST, and EconoFit Profinity eXact Columns.

- The Nuvia IMAC and Profinity IMAC Resins isolate histidine-tagged proteins, the Profinity GST Resin purifies proteins tagged with glutathione S-transferase (GST), and Profinity eXact is an autocleaving resin that provides purification of tag-free proteins



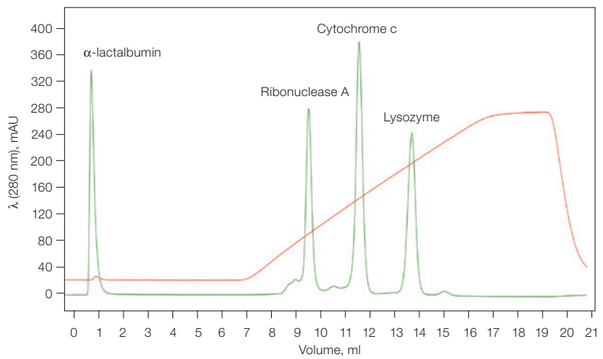
Example of a tagged affinity separation. Most proteins pass through the column (flow-through peak) while the elution peak represents those with a specific attraction to the resin ligand.

Ion Exchange

Ion exchange chromatography separation occurs through a charged protein of interest being attracted to an oppositely charged chromatography resin. Separation of proteins is performed by changing the ionic strength or pH of the solution in a manner that differentially changes the binding properties of proteins interacting with the matrix. Elution protocols include the use of gradients, isocratic steps, or a combination thereof. Ion exchange can be used for protein isolation, intermediate purification, or as a polishing technique.

Bio-Rad offers a variety of high-quality ion exchange chromatography columns with a variety of particle sizes, functional groups, and column chemistries.

- The high-performance and high-capacity UNOsphere and Nuvia Ion Exchange Resins and Columns are designed for capture and intermediate steps of lab-scale protein purification and for bioprocess screening. Their bead size makes them ideal for use with crude samples
- The exceptionally high-resolution ENrich and UNO™ Ion Exchange Resins are capable of resolving the most difficult and complex protein samples and are ideal for the polishing steps of the lab-scale protein purification workflow
- The Macro-Prep™ Ion Exchange Resins and Columns combine high resolution and capacity, providing solutions for any step of protein purification



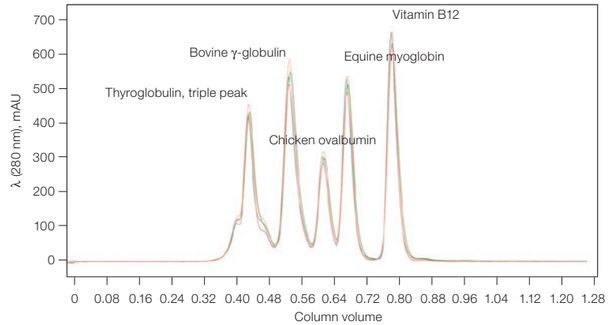
Example of a cation exchange separation of protein standards. The ENrich S Column (1 ml) provides high-resolution separation of a 120 mg maximum protein load with a 0.5–2 ml/min flow rate.

Size Exclusion

Size exclusion chromatography (SEC), also known as gel filtration, is a nonbinding technique that separates proteins based on their size differences. Buffer composition does not typically impact the separation, so the entire method is performed under isocratic conditions. Since low volume loading (on average ≤10%) conditions are required for optimal resolution, this technique is most often used for polishing or protein characterization.

Bio-Rad offers ENrich Columns for the high-resolution separation of proteins, and EconoFit Bio-Gel™ P-6 and Bio-Scale Mini Bio-Gel P-6 Size Exclusion Columns for the efficient desalting and buffer exchange of protein samples.

- ENrich Size Exclusion Columns provide rapid, reproducible high-resolution separations and purification of proteins based on size. These columns can run at high flow rates, which result in exceptionally short separation times
- Bio-Gel P-6 Resin and Columns are used for fast and efficient desalting and buffer exchange of protein samples



Example of a size exclusion separation. The ENrich SEC 650 Column provides the same high resolution for a molecular weight range of 5–650 kD run at high flow rates, including 1.5 and 2.0 ml/min.

Mixed Mode

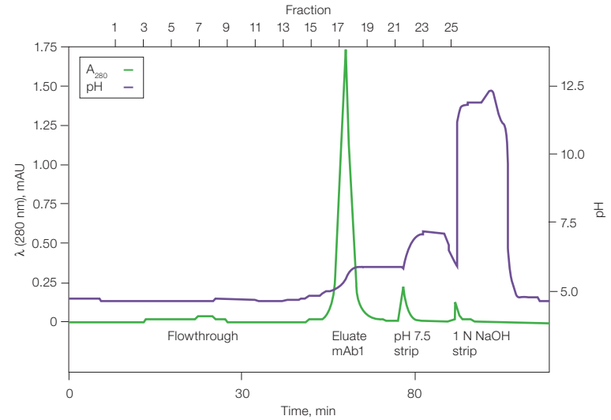
Mixed-mode chromatography is a technique that offers unique separation properties by using multiple separation modalities. Using mixed-mode chromatography as an intermediate purification or polishing step can offer unparalleled selectivity and resolution for a variety of biomolecules.

Bio-Rad offers columns with a variety of mixed-mode resins — perfect for lab-scale or biopharma scouting applications.

- Ceramic Hydroxyapatite (CHT™), Ceramic Hydroxyfluoroapatite (MPC), and Ceramic Fluoroapatite (CFT) Media, with their unique capability to remove Protein A, endotoxin, nucleic acids, and aggregates, are considered industry gold standards for the purification of antibodies and are ideal for the second or third step of an antibody purification workflow
- Nuvia cPrime and aPrime 4A Resins combine hydrophobic interaction and ion exchange modalities to achieve effective purification

Hydrophobic Interaction

- Macro-Prep Hydrophobic Interaction Chromatography Resins are designed specifically for intermediate purification steps that remove host-cell contaminants from partially purified targets



Example of a mixed-mode separation. Nuvia cPrime Resin as the final polishing step of a monoclonal antibody (mAb) purification.

Development to Scale-Up

Bio-Rad has a complete portfolio of proven high-performance chromatography resins that make the transition from lab- to process-scale protein purification seamless. Many of the lab-scale columns are prepacked with resins that are also available in bulk. Be sure to discuss your development and scale-up needs with your Bio-Rad chromatography specialist or visit **bio-rad.com/Process-Scale** for a complete list of Bio-Rad process-scale resins.

Resins Available in Prepacked Columns

Chromatography Mode	Resin	Scale: Lab	Scale: Process	Prepacked Columns
Affinity	Nuvia IMAC	•	•	EconoFit and Foresight
	Profinity IMAC	•		EconoFit
	UNOsphere SUPrA	•		EconoFit
	Affi-Gel and Affi-Prep	•		EconoFit
	Profinity GST	•		EconoFit
	Profinity eXact	•		EconoFit
Ion exchange	Nuvia Q and S	•	•	EconoFit and Foresight
	Nuvia HP-Q	•	•	EconoFit and Foresight
	Nuvia HR-S	•	•	EconoFit and Foresight
	UNO Q and S	•		UNO
	UNOsphere Q and S	•	•	EconoFit and Foresight
	ENrich Q and S	•		ENrich
	Macro-Prep DEAE and CM	•	•	EconoFit
	Macro-Prep High Q and Q-3HT	•	•	EconoFit
	Macro-Prep High S	•	•	EconoFit
Size exclusion	ENrich SEC	•		ENrich
	Bio-Gel P-6	•	•	EconoFit and Bio-Scale Mini
Mixed mode	CHT	•	•	EconoFit and Foresight
	CFT	•	•	EconoFit
	MPC	•	•	EconoFit and Foresight
	Nuvia cPrime	•	•	EconoFit and Foresight
	Nuvia aPrime 4A	•	•	EconoFit and Foresight
Hydrophobic interaction	Macro-Prep Methyl and t-Butyl	•	•	EconoFit

Column Selection Guide

100% Homogeneity

Protein Purity

Antibody Purification Workflow

POLISHING

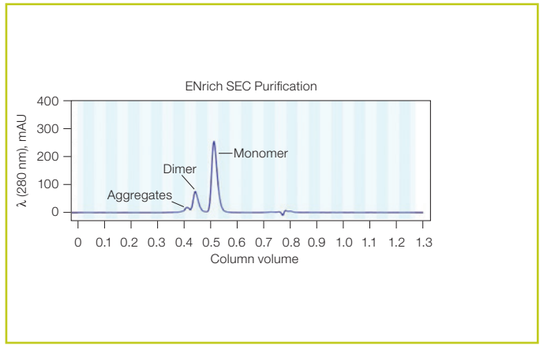
Analytical	ENrich SEC, Aminex™	Aminex Columns provide excellent separation of carbohydrates, organic acids, and alcohols
High Resolution	ENrich Q and S, ENrich SEC, Nuvia HR-S, UNO Q and S, CHT, MPC, CFT, Nuvia cPrime, Nuvia aPrime 4A	Gain high specificity with the mixed-mode modality of CHT, MPC, and CFT Media and Nuvia cPrime and aPrime 4A Resins
Good Resolution, Fast Flow	ENrich SEC, Aminex, Macro-Prep IEX	Macro-Prep Resins offer the flexibility of removing bulk contaminants and trace impurities with high flow rates

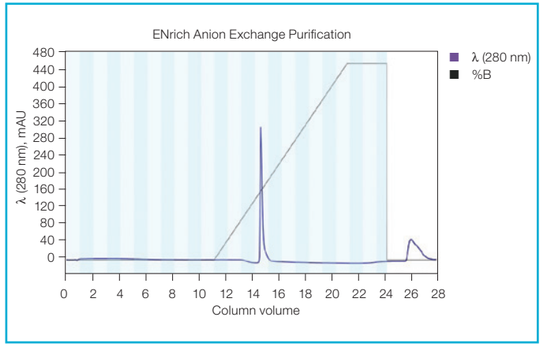
INTERMEDIATE PURIFICATION

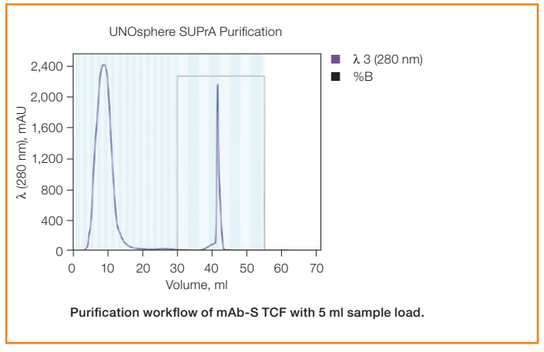
High Resolution	ENrich Q and S, Nuvia HR-S, UNO Q and S	ENrich Resins are ideal for resolution of similar protein species or removal of bulk contaminants and trace impurities
Good Resolution, Fast Flow	Macro-Prep IEX and HIC, UNOsphere Q and S, Nuvia Q and S	Use Macro-Prep, UNOsphere, and Nuvia Resins for capture or intermediate purification

CAPTURE

Tagged Purification	Nuvia IMAC, Profinity GST, Profinity eXact, Profinity IMAC	Use Nuvia and Profinity Resins for high purity of tagged proteins in one step
Antibody Purification	UNOsphere SUPrA, Affi-Prep Protein A, Affi-Gel Protein A, Affi-Gel Blue	UNOsphere SUPrA, Affi-Prep, and Affi-Gel Resins offer high purity of antibodies and antibody fragments in one step







Purification workflow of mAb-S TCF with 5 ml sample load.

Our Family of Products

ENrich Columns

High-resolution ion exchange and size exclusion columns for intermediate and polishing purifications.

Visit bio-rad.com/ENrich for more product details.

EconoFit Columns

Versatile low-pressure columns that are prepacked with a variety of resins. Convenient and compatible with most commonly used chromatography systems.

Visit bio-rad.com/EconoFit for the most up-to-date information and to download product manuals.

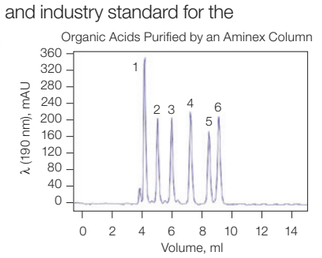
Foresight Columns and Filter Plates

Specially designed column formats intended for scale-up and modeling studies, packed with a range of process-scale resins.

Visit bio-rad.com/Foresight for a complete list of Foresight products.

Aminex HPLC Columns

Aminex HPLC Columns are the research and industry standard for the analysis of carbohydrates, organic acids, and alcohols in food and beverage, biofuel, and biotechnology applications.



High peak resolution of oxalic (1), citric (2), malic (3), succinic (4), formic (5), and acetic (6) acids.

Visit bio-rad.com/Aminex for a complete list of Aminex products.

Our prepacked resins and media are also available in bulk resin format. Visit bio-rad.com/Lab-Scale to view our full selection of lab-scale resins and media.

Column Selection Table

Prepacked Columns	Catalog Number	Quantity	Mean Particle Size, µm	Capacity of Resin*	Feature/Application
Gel Filtration/Desalting					
ENrich SEC 70	7801070	1 x 24 ml	10	250 µl	High resolution, rapid separation of biomolecules up to 70 kD
ENrich SEC 650	7801650	1 x 24 ml	10	250 µl	High resolution, rapid separation of biomolecules up to 650 kD
EconoFit Bio-Gel P-6**	12009238 12009239	1 x 5 ml 5 x 5 ml	90–180	≤22% of column volume	Desalting
Mixed Mode					
EconoFit CHT Type I, 40 µm	12009255 12009253 12009254	1 x 1 ml 1 x 5 ml 5 x 5 ml	40	≥25 mg lysozyme/g CHT; 25–60 mg IgG/ml CHT	High capacity, high selectivity
EconoFit CHT Type II, 40 µm	12009259 12009257 12009258	1 x 1 ml 1 x 5 ml 5 x 5 ml	40	≥12.5 mg lysozyme/g CHT; 15–25 mg IgG/ml CHT	High selectivity
EconoFit CHT Type I, 80 µm	12009256	1 x 1 ml	80	≥25 mg lysozyme/g CHT; 25–60 mg IgG/ml CHT	High capacity, high selectivity
EconoFit CHT Type II, 80 µm	12009260	1 x 1 ml	80	≥12.5 mg lysozyme/g CHT; 15–25 mg IgG/ml CHT	High selectivity
EconoFit CHT XT, 40 µm	12009261	1 x 1 ml	40	17–25 mg lysozyme/g CHT; ≥60 mg mAb G/ml CHT	Unique selectivity
EconoFit CFT Type II, 40 µm	12009252 12009240 12009251	1 x 1 ml 1 x 5 ml 5 x 5 ml	40	14–21 mg lysozyme/ml; 33 mg IgG/ml CFT	Acidic proteins
EconoFit MPC Type I, 40 µm	12009279	1 x 1 ml	40	≥25 mg lysozyme/g MPC; 25–50 mg IgG/ml MPC	Unique selectivity
EconoFit Nuvia aPrime 4A	12009280	1 x 1 ml	50	≥50 mg acidic mAb/ml	Hydrophobic anion exchanger; high recovery, unique selectivity
EconoFit Nuvia cPrime	12009281	1 x 1 ml	70	>40 mg human IgG/ml; >60 mg lactoferrin/ml	Hydrophobic cation exchanger; high recovery, unique selectivity
Anion Exchange					
ENrich Q	7800001 7800003	1 x 1 ml 1 x 8 ml	10	130 mg BSA 1,000 mg BSA	Strong anion exchanger; high resolution, rapid separation
UNO Q	7200001 7200003	1 x 1.3 ml 1 x 6 ml	Monolith	20 mg BSA 90 mg BSA	Strong anion exchanger; high-resolution separation at high flow rates
EconoFit Macro-Prep DEAE	12009274 12009264 12009265 12009266	1 x 1 ml 5 x 1 ml 1 x 5 ml 5 x 5 ml	50***	≥30 mg BSA/ml	Weak anion exchanger; different selectivity than strong anion exchangers
EconoFit Macro-Prep High Q	12009275 12009267 12009268 12009269	1 x 1 ml 5 x 1 ml 1 x 5 ml 5 x 5 ml	50***	≥37 mg BSA/ml	Strong anion exchanger; good to high resolution for preparative separations, high capacity
EconoFit Macro-Prep High Q-3HT	12009283	1 x 1 ml	50***	≥37 mg BSA/ml	Similar to Macro-Prep High Q, but with different pore size characteristics
EconoFit UNOsphere Q	12009307 12009301 12009302 12009303	1 x 1 ml 5 x 1 ml 1 x 5 ml 5 x 5 ml	120	≥180 mg BSA/ml	Strong anion exchanger; efficient capture from crude extracts
EconoFit Nuvia Q	12009290	1 x 1 ml	85	>170 mg BSA/ml	Strong cation exchanger; ultra-high capacity
EconoFit Nuvia HP-Q	12009282	1 x 1 ml	50	>50 mg thyroglobulin/ml	High capacity; ideal for purification of large biomolecules
Cation Exchange					
ENrich S	7800021 7800023	1 x 1 ml 1 x 8 ml	10	120 mg human IgG 940 mg human IgG	Strong cation exchanger; high resolution, rapid separation
UNO S	7200021 7200023	1 x 1.3 ml 1 x 6 ml	Monolith	20 mg IgG 90 mg IgG	Strong cation exchanger; high-resolution separation at high flow rates
EconoFit Macro-Prep High S	12009276 12009270 12009271 12009272	1 x 1 ml 5 x 1 ml 1 x 5 ml 5 x 5 ml	50***	≥49 mg human IgG/ml	Strong cation exchanger; good to high resolution for preparative separations, high capacity
EconoFit Macro-Prep CM	12009273	1 x 1 ml	50***	≥25 mg hemoglobin/ml	Weak cation exchanger; different selectivity than strong cation exchangers
EconoFit UNOsphere S	12009308 12009304 12009305 12009306	1 x 1 ml 5 x 1 ml 1 x 5 ml 5 x 5 ml	80	60 mg human IgG/ml	Strong cation exchanger; efficient capture from crude extracts
EconoFit Nuvia S	12009291	1 x 1 ml	85	>110 mg human IgG/ml	Strong cation exchanger; ultra-high capacity
EconoFit Nuvia HR-S	12009284	1 x 1 ml	50	>70 mg human IgG/ml	High-resolution, strong cation exchange resin; separation of closely related biomolecules
Hydrophobic Interaction					
EconoFit Macro-Prep Methyl	12009277	1 x 1 ml	50***	15 mg HSA/ml	Intermediate purification steps; for biomolecules with strong hydrophobic regions
EconoFit Macro-Prep t-Butyl	12009278	1 x 1 ml	50***	25 mg HSA/ml	Intermediate purification steps; for biomolecules with few or weak hydrophobic regions
Tagged Affinity					
EconoFit Nuvia IMAC	12009288 12009285 12009286 12009287	1 x 1 ml 5 x 1 ml 1 x 5 ml 5 x 5 ml	50	≥40 mg histidine-tagged protein/ml	Histidine-tagged proteins; ultra-high capacity
EconoFit Nuvia IMAC, uncharged	12009289	1 x 1 ml	50	≥40 mg histidine-tagged protein/ml when charged	Customized metal ion charging
EconoFit Profinity IMAC	12009298 12009299 12009300	5 x 1 ml 1 x 5 ml 5 x 5 ml	60	≥15 mg histidine-tagged protein/ml	Histidine-tagged proteins; high purity for a wide molecular weight range
EconoFit Profinity GST	12009295 12009296 12009297	5 x 1 ml 1 x 5 ml 5 x 5 ml	70	≥11 mg GST-tagged protein/ml	GST-tagged proteins
EconoFit Profinity eXact	12009292 12009293 12009294	1 x 1 ml 5 x 1 ml 1 x 5 ml	60–160	>3 mg tag-free maltose binding protein/ml	On-column cleavage of proteins tagged with subtilisin prodomain
Immunoglobulin Affinity					
EconoFit UNOsphere SUPrA	12009321 12009322 12009323	1 x 1 ml 5 x 1 ml 1 x 5 ml	53–61	>20 mg polyclonal human IgG/ml	Antibody purification; recombinant Protein A, high capacity
EconoFit Affi-Prep Protein A	12009236 12009237	5 x 1 ml 1 x 5 ml	61	8–10 mg mouse IgG1/ml 16–23 mg human IgG/ml	Purification of a range of monoclonal and polyclonal antibodies
EconoFit Affi-Gel Blue	12009234 12009235	1 x 5 ml 5 x 5 ml	80–150	>11 mg albumin/ml	Albumin removal and blood protein purification
EconoFit DEAE Affi-Gel Blue	12009262 12009263	1 x 5 ml 5 x 5 ml	150–300	0.2–1 ml serum/ml gel; IgG recovery >55%, albumin recovery >90%	IgG purification from serum; alternative to Protein A chromatography

* See product manuals for specific measurement conditions. ** Bio-Gel P-6 Columns are also available in 10 and 50 ml sizes. Visit bio-rad.com/BSM-P6-Columns for more details. *** Before derivatization.

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Visit **bio-rad.com/Columns** to view our full selection of prepacked and empty columns.

Visit **bio-rad.com/Process-Scale** to learn about our process-scale resins, media, and columns.

Nuvia aPrime 4A Resin is covered by U.S. Patent Number 9,669,402 and foreign counterparts.

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Bio-Rad Laboratories, Inc.

Life Science Group

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