Process-Scale Chromatography
Your Key to a Successful Journey

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Chromatography: Your Key to a Successful Journey

The journey of a drug to market begins with a molecule that shows promise to treat, cure or prevent disease. There are many steps along this journey; one of the most important is the need to separate and purify your molecule from a complex feedstream.

As a drug manufacturer, you are under pressure to provide robust solutions faster and at lower cost. With changing manufacturing requirements, chances are you are experiencing challenges that can affect the journey of your drug.

Chromatography is your answer to getting high purity products. EMD Millipore offers a complete portfolio of chromatography resins, membrane adsorbers, buffers, Cleaning in Place (CIP) solutions and compatible hardware. Coupled with proven expert support, we provide you with the confidence you need to tackle any chromatography challenge in your downstream process.

Did You Know?

Just one year after the discovery of the chromatographic principle in 1903, EMD Millipore began manufacturing the first chromatography products. Since then, we've continued to evolve, focusing strongly on R&D to provide you with chromatography solutions that best suit your needs.

Determine the Right Chromatography Technique

Depending on your process parameters, you have options when implementing a chromatography technique, utilizing membrane and/or bead-based technologies that remove key impurities. However, with a diverse landscape of molecules, it can be difficult to determine which purification approach to take.

We partner with you every step of the way, offering you guidance through our full suite of purification solutions that enable you to deliver on your objectives.

Molecule Type	Chromatography Approach
Monoclonal antibody	Affinity Ion exchange Hydrophobic interaction
Recombinant protein	Ion exchange Reversed phase Affinity Hydrophobic interaction Size exclusion
Plasma protein	Ion exchange Size exclusion
Vaccine	Ion exchange Size exclusion
Peptide	Reversed phase Ion exchange
Small molecule	Reversed phase

Technique:
Affinity Chromatography

At the capture phase of purification, the primary goals are to isolate the target product as quickly as possible from the clarified feedstock, reduce the process volume to a manageable scale for subsequent steps, and maximize yield and economy.

Affinity chromatography, a method of separating biochemical mixtures based on a highly specific interaction, can effectively achieve these goals. This approach is designed to purify and concentrate substances from a feedstream into a buffering solution and to reduce the volume for subsequent processing.

Fractogel® EMD Chelate Resin

For Fractogel® EMD Chelate resin, iminodiacetic acid has been chosen as the affinity ligand. Various metal ions can be immobilized on the stationary phase via this immobilized chelating agent. Free coordination sites of the metal ions are used to bind different proteins and peptides.

ProSep® Ultra Plus Resin

This protein-A based affinity chromatography media is designed for large-scale, cost-effective purification of high titer therapeutic antibodies. A smaller particle size, with optimized pore size and ligand immobilization, has resulted in a significant increase in dynamic capacity. Its rigid base matrix allows for predictable scale-up and greater flexibility, enabling you to reduce your equipment footprint and purify larger volumes of feedstock with minimal time.

Technique:
Ion Exchange Chromatography

The most widely used method to produce a pure protein product is **Ion Exchange (IEX) Chromatography**, a process that separates molecules based on their charge.

Proteins are very complex molecules that can have positively or negatively charged surface areas. IEX media have charged functional groups that are able to bind molecules of the opposite charge. By choosing the right chromatographic conditions (e.g. salt concentration, buffer system and pH), proteins can be separated. In common practices, bound molecules are selectively eluted by application of an increasing concentration of a monovalent salt.

The Fractogel® Family of Resins

Synthetic polymer media, with proprietary tentacle technology, offers a number of advantages over conventional resins. Long linear polymer chains increase the functional groups' accessibility for biomolecules, ensuring a tighter binding of target substances and sharp elution profiles. The result: high throughput and high selectivity with excellent purity and yield.

The Eshmuno® Family of Resins

A unique family of ion exchangers designed for highly productive downstream purification of monoclonal antibodies (mAbs). Based on the proven tentacle technology, its rigid base beads enable easy packing. With high binding capacity and excellent pressure-flow behavior, Eshmuno® resins are the most efficient resins available for host cell proteins (HCP) removal. With Eshmuno® resins, you are able to reduce time and manufacturing costs, resulting in outstanding productivity.

ChromaSorb™ Membrane Adsorber

An innovative, flow-through anion exchange membrane that is designed to remove trace impurities – including HCP, DNA, endotoxins, and viruses. This device provides the greatest levels of impurity binding at the highest salt concentrations for mAbs and protein purification. ChromaSorb™ membrane adsorber provides robust clearance with no dilution of your feedstream, resulting in significant savings on buffer usage, improved process economics and manufacturing plant flexibility.

Technique: Reversed Phase Chromatography

Reversed Phase (RP) Chromatography is a method for the purification of recombinant proteins, therapeutic peptides and small molecule APIs. This technique separates molecules based on interaction with a hydrophobic matrix, largely based on their polarity. Molecules bind to a hydrophobic matrix in an aqueous buffer (polar) and elute from the matrix using a gradient of organic solvent (non-polar). This technique is often used as a final polishing step and is recommended for purity checking and protein mapping.

LiChroprep® Sorbent

An irregular shaped silica gel for advanced normal-phase and reversed-phase chromatography. Its narrow, well-defined particle size distribution offers high performance, permeability and good selectivity. Used for polishing applications in biopharmaceutical processes.

PharmPrep® P Sorbent

A spherical, porous silica carrier with a uniform and homogenous gel matrix for preparative liquid chromatography. The particles have a perfect spherical shape, and are available in 10 and 20 µm particle sizes. PharmPrep® P Sorbent fits perfectly into polishing steps of recombinant proteins and therapeutic peptides such as insulin and other molecules. Excellent batch-to-batch reproducibility and high productivity of your purification process can be achieved.

Technique: Hydrophobic Interaction Chromatography

High performance hydrophobic interaction chromatography (HIC) is a powerful technique for the purification of proteins at production scale.

The method of HIC is based on the interaction between hydrophobic ligands fixed on the chromatographic support and hydrophobic areas located on the surface of proteins. The hydrophobic feature of a given protein is caused by certain amino acid residues such as isoleucine, valine, leucine, phenylalanine, and others. Although most of the hydrophobic amino acid residues are located in the center of the molecule, hydrophobic patches can be found on the protein's surface as well. High salt concentrations lead to a better separation of the target protein from the impurities.

Fractogel® EMD Propyl Media and Fractogel® EMD Phenyl Media

Both chromatography media contain tentacle surface modification displaying unique hydrophobic features. Their high selectivity allows for efficient purification in a wide range of applications. The main application areas for HIC are the purification of cytosolic proteins, antibodies and recombinant proteins. Membrane proteins can also be isolated with this technique. Additionally, HIC is suited for the removal or exchange of non-ionic detergents. Both media are mechanically stable and resistant to biological and chemical degradations. With a particle size of 20-40 µm, Fractogel® EMD Phenyl media is the material of choice for purification of proteins with a large amount of hydrophobic areas. Due to the weak hydrophobicity of Fractogel® EMD Propyl media, a high mass recovery can often be achieved.

Technique: Size Exclusion Chromatography

Size exclusion chromatography (SEC) is the method of choice for polishing of recombinant proteins, viruses and plasma-derived biotherapeutics.

Fractogel® EMD BioSEC Media

Fractogel® EMD BioSEC media exhibits higher flow rates compared to known soft gels, which supports column regeneration and improves process economics. Its excellent pressure stability and mechanical strength make the columns easy to pack and supporting high purification throughput. The high selectivity and its tentacle structure leads to excellent recoveries and a highly pure product.

Choosing the Right Components

Buffers

Buffers used in the downstream process are a critical component of chromatography as they are used to provide a defined environment for maintaining biomolecules in a stable native form with full activity. They are also key for the elution of the product of interest and used to condition the resin for next processing steps.

EMD Millipore is a leading supplier of buffers and salts that work seamlessly with our chromatography resins. To ensure protection of the target protein, these buffers are manufactured under GMP conditions in order to minimize bioburden and endotoxin levels. Comprehensive regulatory documentation is available in the EMPROVE® bio dossiers.

Cleaning in Place

Every year, biopharmaceutical production facilities use vast quantities of Cleaning in Place (CIP) solutions. And every year, more and more companies order the custom-made products they need from EMD Millipore, rather than preparing them themselves. Why? Because they know from experience that our top quality, ready-to-use products can save time and money.

To ensure the highest standards of purity and consistent quality, all our CIP solutions are produced in a clean room at our new GMP process solutions plant in Darmstadt, Germany. We use only pharmaceutical-quality raw materials and highly purified water according to European and US pharmacopoeia. As a result, you can integrate our solutions directly into your own clean room without further ado. Since all our CIP products comply with GMP guidelines, you are well prepared for the future in this increasingly regulated segment.

Buffer Recommendations

Most chromatography applications take place in the presence of aqueous buffer systems to avoid protein denaturation. Positively charged buffering ions should be used on anion exchangers. For cation exchangers, the buffering ion should be negatively charged.

Chromatography Columns and Systems

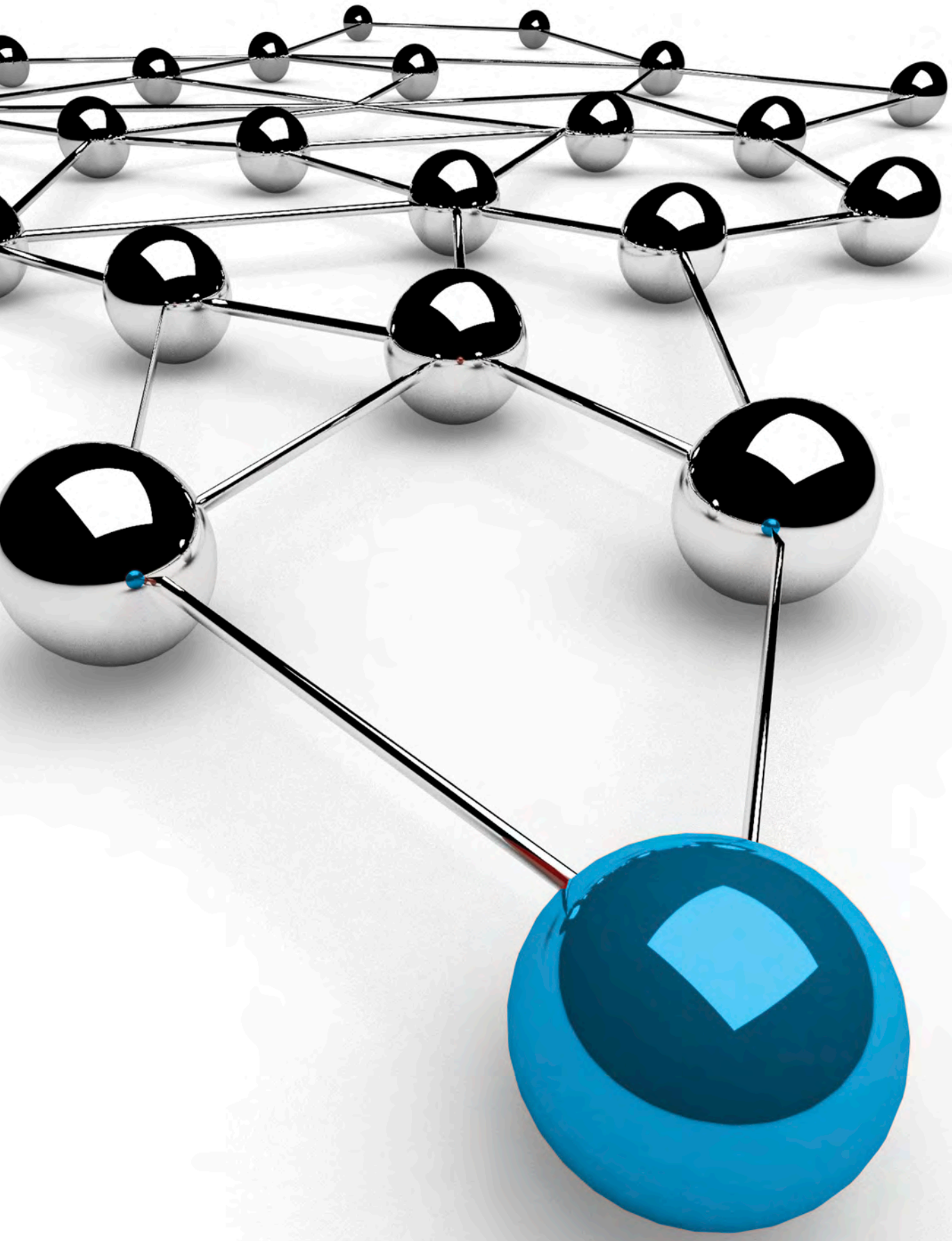
Chromatography columns and systems are critical factors to the successful separation of your valuable molecule. EMD Millipore provides standard and custom columns and systems from lab-scale to pilot and process-scale. From screening to large-scale production, our columns, systems and single-use solutions are designed to provide robust, consistent performance with the processing flexibility you need.

- Mobius® FlexReady Solution
  This easy-to-use system features a smart, single-use flowpath delivering robust reproducibility, elimination of carryover from previous batches and full automation for the production of small quantities of high-value product faster and with greater reliability. This system supports multi-product, multi-scale production, maximizing processing flexibility while removing processing bottlenecks, reducing risk, and accelerating molecules through the development and clinical manufacturing pipeline.
- K-Prime® Systems
  Automated systems controlled by CCP® platform (CFR21.11 compliant) for developing bio-molecule separations requiring flow rates from 20 mL to 10 L per minute. This scalable family of systems delivers sharp separations in applications from small-scale pilot processes through commercial production.
- Vantage® Columns
  From initial screening through pilot to small-scale production, these columns can be axially packed in manual or automated mode. They are ideally suited to the newer and more rigid chromatographic media applications.
- QuikScale® Columns
  From 70 mm to 630 mm column sizes, this family of columns delivers greater product purity at high linear velocities for maximum productivity. These robust columns are easy to pack/unpack to deliver optimal resolution across a range of applications, accommodating all media types.

- IsoPak® Columns
  Available from 440 mm to 1400 mm, these production-scale columns provide faster, cleaner and more consistent packing and unpacking, and deliver reproducible chromatographic performance.
- HIBAR® Columns
  Pre-packed, ready-to-use columns that can easily be connected to any HPLC system. With an internal diameter of 25 mm or 50 mm, this size is convenient for separations of mg up to grams range of final product.
- RoboColumns and MiniChrom columns
  For parallel analytical and preparative chromatography with small volumes and rapid separation. Filled with ProSep®, Fractogel® and Eshmuno® media for High Throughput Screening (HTS). RoboColumns are easy-to-use, pre-packed mini columns that can be arranged on well plates for laboratory robot workstations.
- ChromaSorb™ Holder
  The ChromaSorb™ holder is designed to securely hold up to two ChromaSorb™ 50 or 500 mL devices running in parallel or sequential mode. Available as either a benchtop unit or with a stand to enable portable free-standing operation. The holder provides safe, robust fluid flow through the ChromaSorb™ devices ensuring proper wetting and increased convenience to the operator. Predefined standard Flexware® single-use assemblies optimized for use with the holder and ChromaSorb™ device have been developed and are available to support greater process flexibility, reliability and speed to implementation.

Parameters affecting the quality of your separation

- Type and quality of chromatography material
- Buffer system
- Column dimension
- Column packing quality
- Elution method (gradient or step), elution buffer
- Gradient and volume
- Flow rate & flow distributor design



Partner with a Leader in Chromatography

EMD Millipore boasts the industry's largest support system, with global facilities, unparalleled expertise and a dedicated service organization aimed at fully supporting your chromatography efforts from molecule to market.

Your Objectives. Our Solutions.

We understand your objectives: improved productivity, accelerated time-to-market and product robustness. That's why we partner with you to:

- Select the right media depending on your process parameters
- Enable process scaling, optimization and yield improvement

Additionally, look to us for:

- Process monitoring, quality control and technical support
- Supply validation and full regulatory documentation
- Chromatography theory, use and best practices training

Our global team of engineers and scientists provide expertise and peer-to-peer support in process development and manufacturing. Whether at your site or in our laboratories, our customized services will help solve your most difficult challenges.

- Access® Compliance and Validation Services
  Our state-of-the-art labs provide customized service packages for scaling, validation and testing.
- Process Systems and Instruments
  Our Field Service Engineers provide expertise to set up and validate your chromatography system quickly.
- Knowledge Institute
  Our experts actively engage attendees in the learning process, teaching real-world solutions and current best practices.
- Application Services
  Our wide range of application services optimize your process, minimize risk, and deliver solutions using current best practices.

Be part of ChromaTALK™!

ChromaTALK™ is an online chromatography community hosted by EMD Millipore. This open platform invites all chromatographers to interact with other industry peers and leading experts in the field of process-scale chromatography. Hear their perspectives on what's new, what's needed, and where the field is headed.

Join the conversation at: **www.chromatalk.com**

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