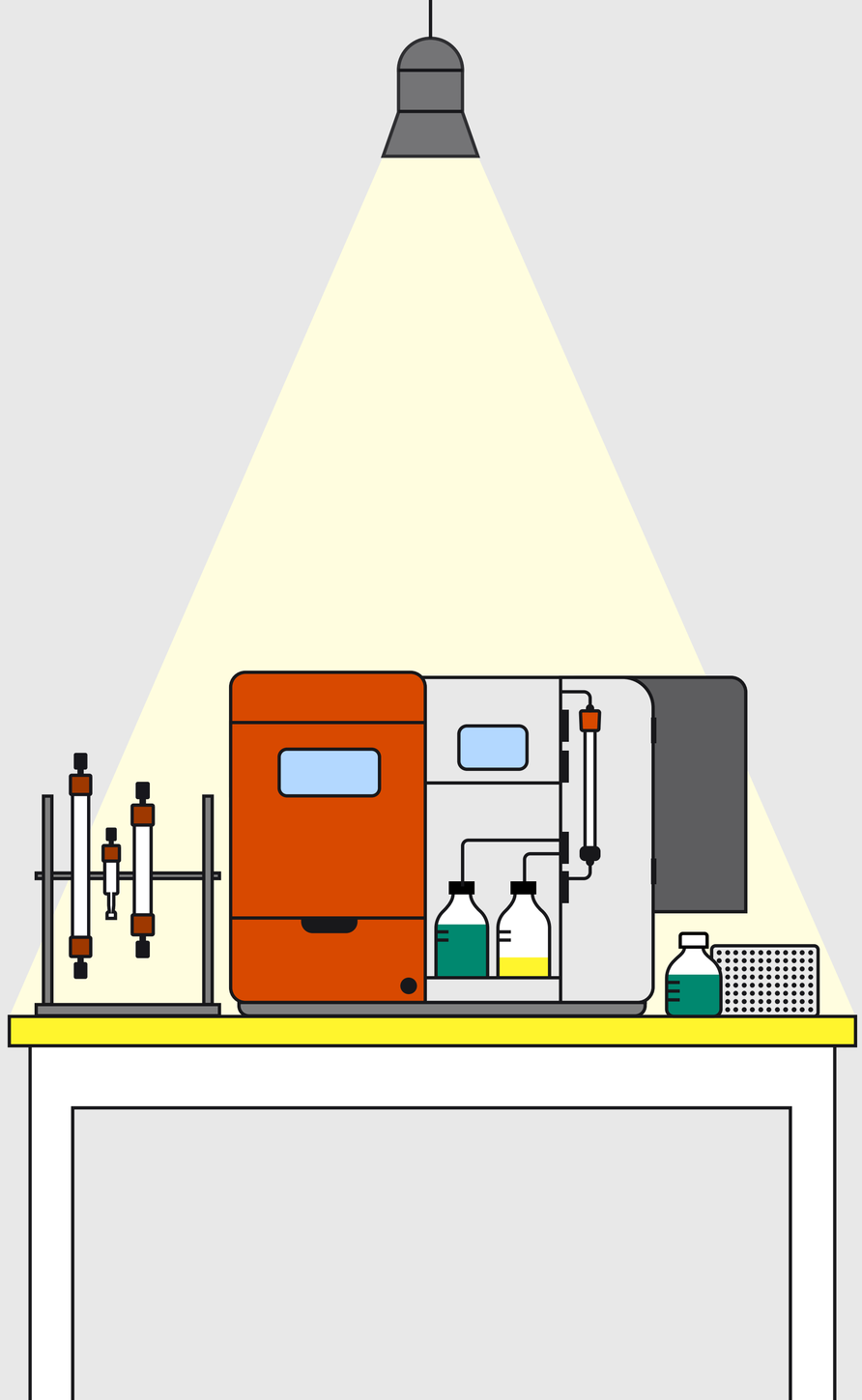
eBook

Spotlight on chromatography

Protein research mini-guides



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Introduction

Protein purification for research: a roadmap

Purifying your target protein is a crucial, and complex, step in protein characterization. And with the wide range of chromatography tools and techniques available today, it's easy to get bogged down researching protocols and products -- time you might prefer to spend generating data.

Use this eBook as a quick guide to considerations for chromatographic protein purification, including basic principles, protocols, and selection charts to help you find the right resin for your molecule.

Chromatography principles

What determines a successful purification scheme?

The output of a protein purification scheme is traditionally described in terms of purity, homogeneity, and yield. Optimization of one of the output parameters can usually only be achieved at the expense of the other output parameters, and each purification step will therefore be a compromise.

Example of output parameters

Purity measures:

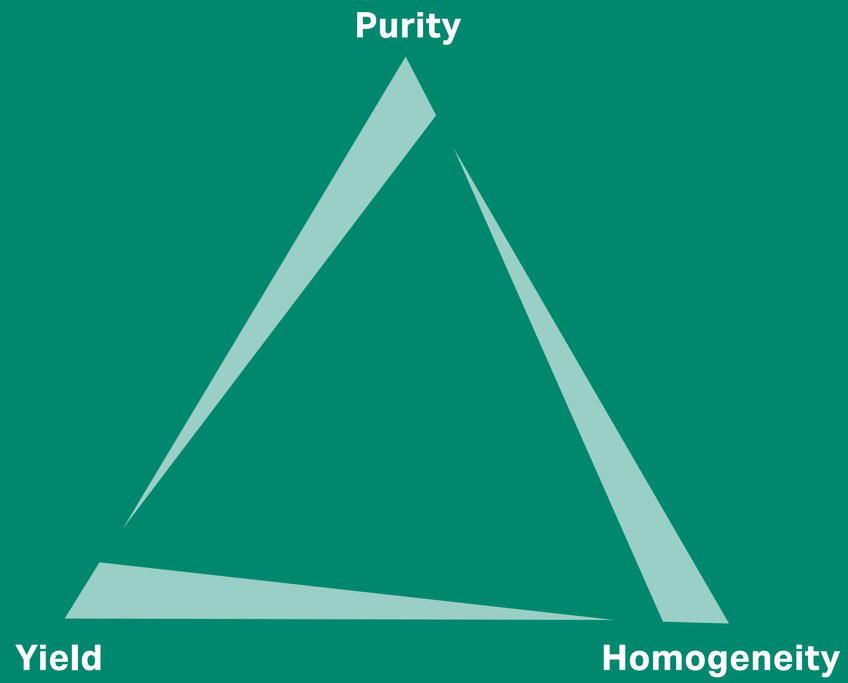
- Electrophoresis, specified as target protein-to-total protein ratio.
- Size exclusion chromatography.

Homogeneity measures:

- Size exclusion chromatography, specified as monomer-to-aggregate content.
- Native electrophoresis.

Yield measures:

- Absorption at 280 nm and using the extinction coefficient for the target protein.
- Activity assay, specified as U/L.
- Concentration using surface plasmon resonance or immune-based assays.



What phases constitute a chromatographic step?

Each chromatographic step can be split into several phases, such as sample load, wash, and elution. Each phase involves different factors (such as pH, conductivity, additives, and flow rate). All have a significant effect on the outcome of the chromatographic step.

What chromatography methods can I choose from?

Chromatographic methods depending on the properties of your target protein and the properties of other substances in the sample.

Protein property	Method
Specific ligand recognition	Affinity chromatography (AC)
Metal ion binding	Immobilized metal ion affinity chromatography (IMAC)
Charge	Ion exchange chromatography (IEX)
Size	Size exclusion chromatography (SEC)
Hydrophobicity	Hydrophobic interaction chromatography (HIC) Reversed phase chromatography (RPC)
Combination of several protein properties, such as charge, size or hydrophobicity	Multimodal chromatography (MM)

How do I select a chromatography resin?

Purification efficiency is dependent on the chromatography resin selected for each technique. The particle size of the resin strongly affects efficiency and flow resistance. A resin with large beads gives chromatography columns with low resolution (broad peaks), but generates low backpressure. Small beads give higher resolution (narrow peaks), but generate higher backpressure.

A general principle of choosing chromatography resins is a larger bead size for early purification steps, and smaller bead size for later steps, where demand on purity is increased.

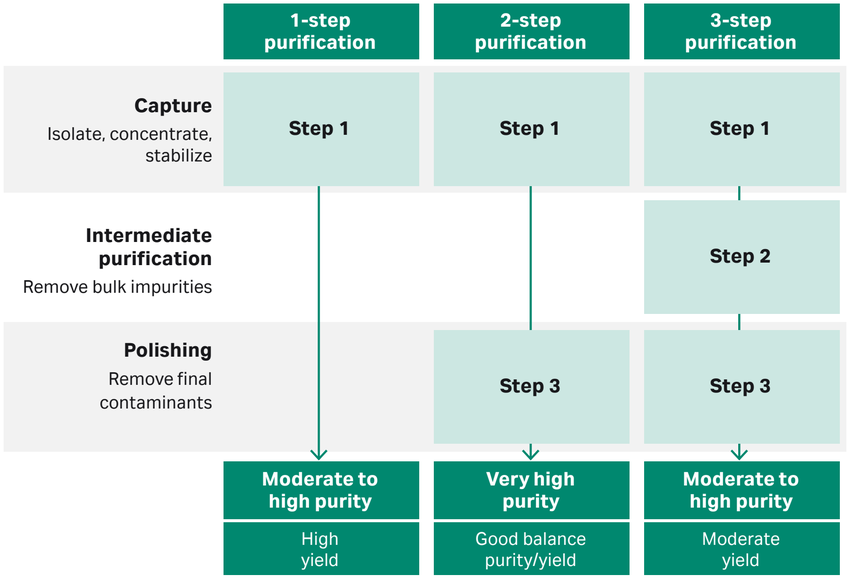
What are the steps in a protein purification protocol?

CiPP (capture, intermediate purification, polishing) purification strategy can be used to structure your purification protocol:

- The initial capture stage isolates, concentrates, and stabilizes your protein.
- Intermediate purification removes bulk contaminants.
- The final polishing step removes the most difficult impurities, such as aggregates of the target protein.

In a research setting, purity requirements are often less important than yield and you may therefore only need a chromatography capture step to achieve your goal.

A general rule is the higher protein purity you need, the more purification steps you will have to use in your workflow. However, additional chromatography steps increase purity, but decrease yield of active protein.



Quickly select the resin for your research:

Guide to chromatography resins for research applications

Click on the chromatography method you are interested in to jump to the corresponding selection guide.

AC
Affinity chromatography
Recombinant tagged proteins

AC
Affinity chromatography
Antibodies and antibody fragments

SEC
Size exclusion chromatography

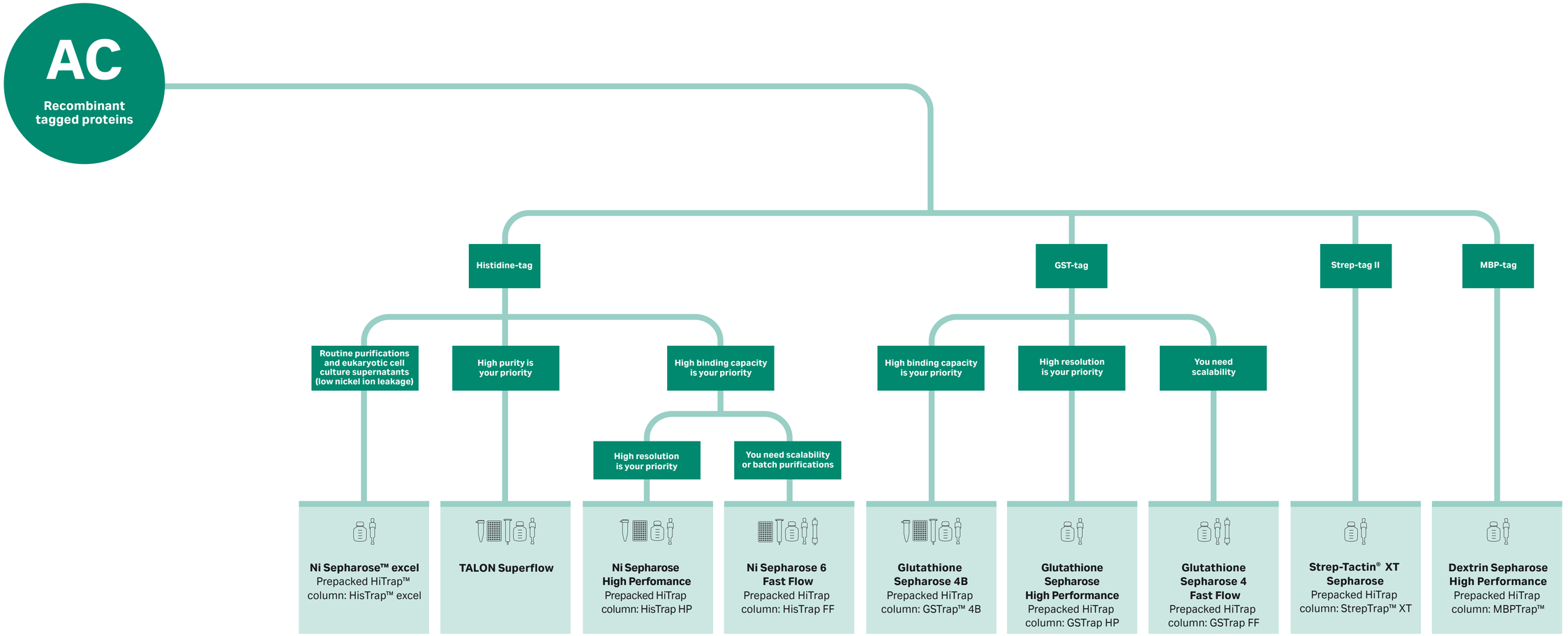
IEX
Ion exchange chromatography

Format guide

	Manual purification						Manual or automated (system) purification				System purification			
Format		Spin columns		96-well plates		Gravity-flow columns		Bottles of chromatography resins		Small prepacked column		Fiber chromatography units		Other columns
Product name		SpinTrap™		MultiTrap™ and PreDictor™ plates		GraviTrap™, MiniTrap™, MidiTrap™, and PD-10		Lab pack		HiTrap™		HiTrap Fibro™ and HiScreen Fibro		HiScreen™, HiPrep™, HiLoad™, RESOURCE™, Tricorn™, and Precision
Application		Screening and quick desalting of small sample quantities using a benchtop centrifuge		High-throughput screening and small-scale purification using centrifuge or vacuum equipment		Simple one-step purification of proteins or sample desalting without the need for equipment		Batch purification and packing chromatography columns		Easy to use with a syringe, peristaltic pump, or a chromatography system		Very high flow rates		Larger scale or high-performance applications

Affinity chromatography for recombinant tagged proteins

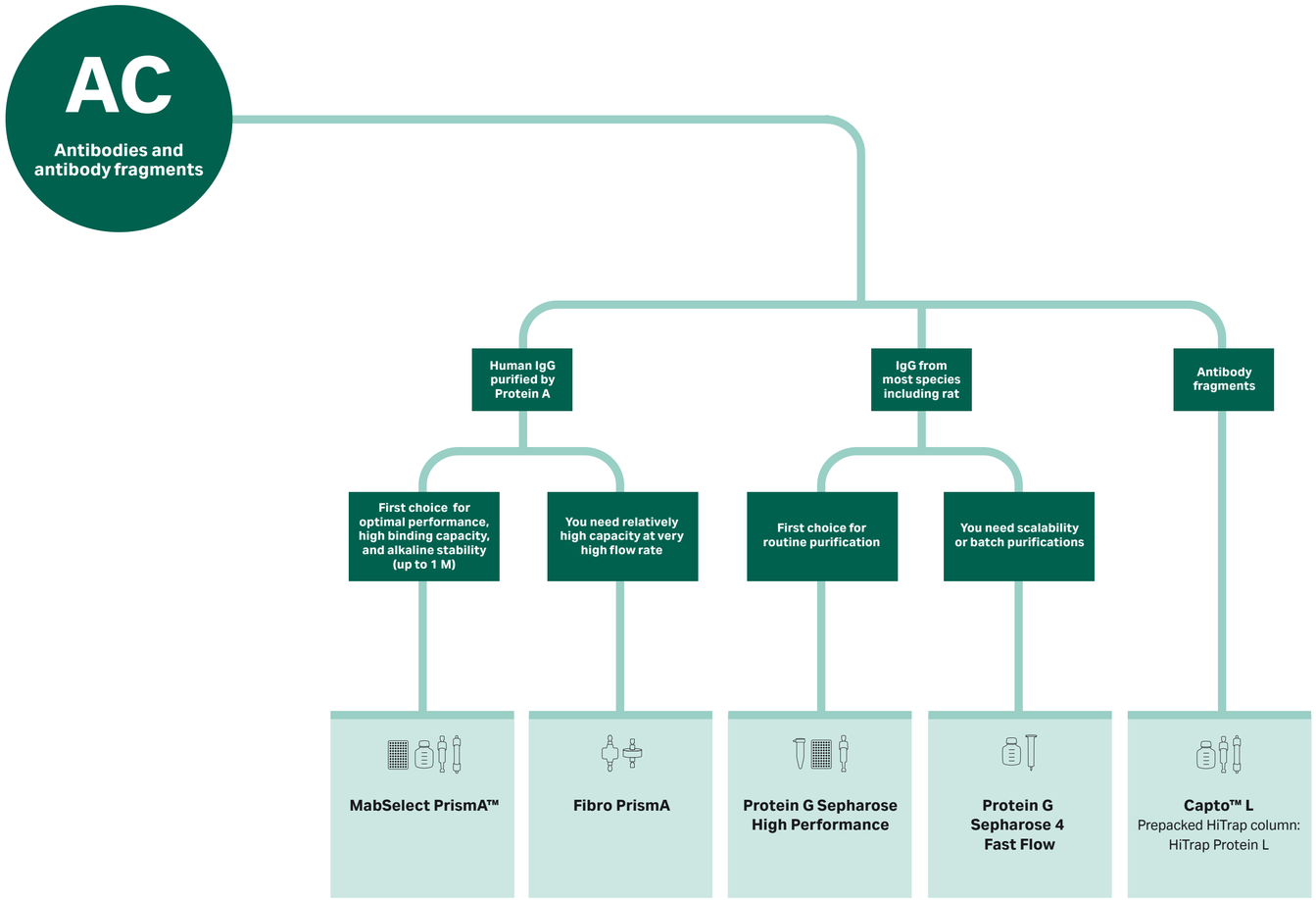
Click on the product names to visit the corresponding web page.



For ordering information, visit **cytiva.com**

Affinity chromatography for antibodies and antibody fragments

Click on the product names to visit the corresponding web page.



Upgrade your resin

If your process uses	Consider	Application
Protein A Sepharose High Performance	MabSelect PrismA	Human IgG purified by Protein A
rProtein A Sepharose Fast Flow	MabSelect PrismA	Human IgG purified by Protein A
MabSelect SuRe™	MabSelect PrismA	Human IgG purified by Protein A
MabSelect SuRe LX	MabSelect PrismA	Human IgG purified by Protein A

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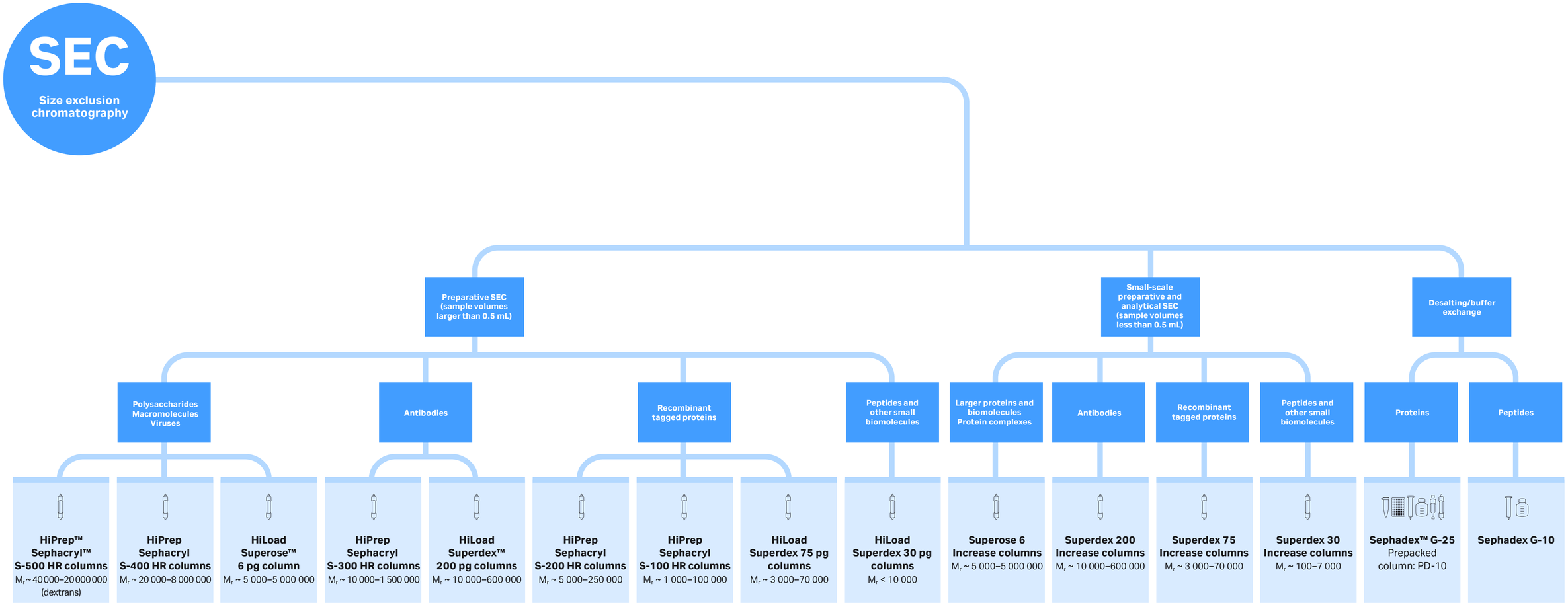
Size exclusion chromatography

Click on the product names to visit the corresponding web page.



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Ion exchange chromatography

Click on the product names to visit the corresponding web page.

IEX
Ion exchange chromatography

Preparative purification

First choice for routine purifications including faster loading of viscous samples (e.g., *E. coli* lysates)

Capto ImpRes
Q (anion exchanger) and SP (cation exchanger)

High resolution and fast flow rates

SOURCE™ 15
Prepacked column: RESOURCE™ Q (anion exchanger) and S (cation exchanger)

Small-scale preparative or analytical purification

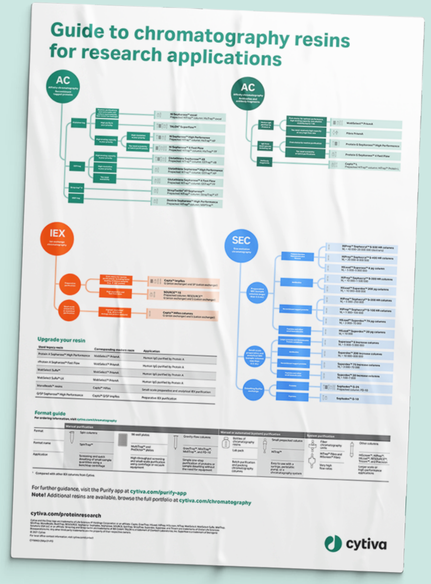
Highest resolution[1] (e.g., structural biology and protein analysis)

Capto HiRes columns
Q (anion exchanger) and S (cation exchanger)

[1] Compared with other IEX columns from Cytiva.

Poster

Download your free guide for your lab wall here.



Upgrade your resin

Used legacy resin	Corresponding modern resin	Application
MonoBeads™ resins	Capto HiRes	Small-scale preparative and analytical IEX purification
Q/SP Sepharose High Performance	Capto Q/SP ImpRes	Preparative IEX purification

ÄKTA™ purification systems

ÄKTA chromatography systems are designed for purification of biomolecules, providing speed and flexibility in research and process development. ÄKTA systems use intelligent UNICORN™ system control software to combine simplicity with power in protein purification from your lab bench to process development and full-scale bioprocess production. Scientists all over the world rely on ÄKTA systems for their protein purification needs.

Configure your system and choose your resins with Purify, our interactive app **here.**

Applications	ÄKTA start™ Transition from manual to automated protein purification/education in protein purification	ÄKTA go™ Achieve desired purity with ease in routine purifications—make the most of valuable bench/ cold-room space	ÄKTA pure™ Flexibility in research—match most current and future purification challenges	ÄKTA avant™ Productivity in process development—fast and secure development of purification processes
Automated and reproducible protein purification including support for gradient elution	•	•	•	•
Support for affinity chromatography, ion exchange chromatography, and multimodal (mixed mode) chromatography	•	•	•	•
Support for size exclusion chromatography	Limited capabilities	•	•	•
Support for hydrophobic interaction chromatography		Limited capabilities	•	•
Software compatible with regulatory requirements, e.g., GLP, GMP		•	•	•
Automated buffer preparation including pH scouting				•
Scale-up, process development			Optional	•
Method development and optimization using design of experiments (DoE)			Optional	•
Automated resin or column scouting			Optional	•
Automated multistep purification			Optional	Optional
Advanced automation including external equipment			Optional	Optional
Recommended flow rate (mL/min)	0.5–5.0	0.01–25.0	0.001–25/0.01–150	0.001–25/0.01–150
Max. operating pressure (MPa)	0.5	5	20/5	20/5

More resources

Whether you're new to protein research or aiming to improve your workflows and results, we're here to help. Click the links in this section to access more information, sign up for our newsletter, or view our eLearning courses.

You can also find a full-length eBook on protein characterization **here.**

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