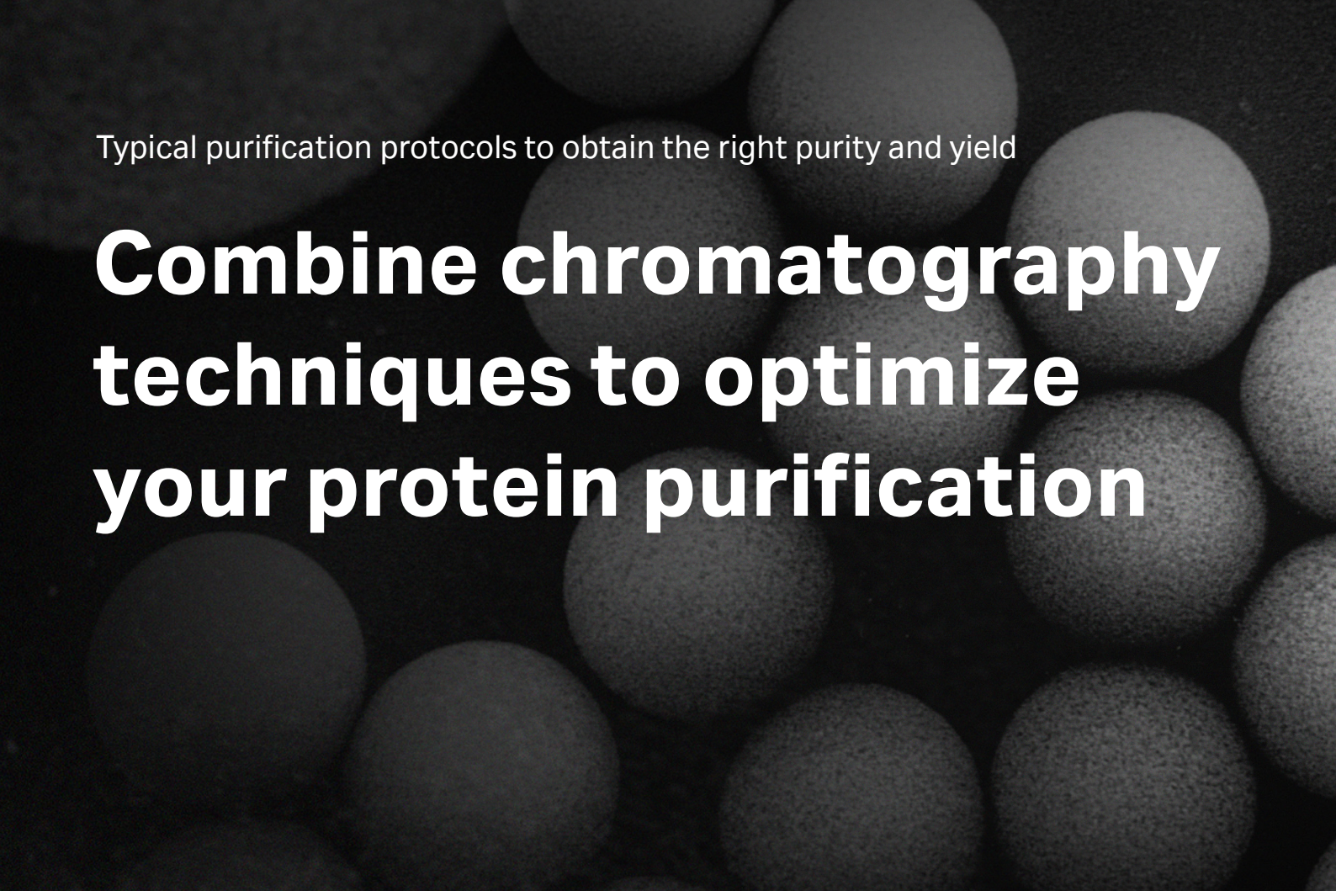


Typical purification protocols to obtain the right purity and yield

Combine chromatography techniques to optimize your protein purification

cytiva

This Quick guide will help you to design a purification protocol in a structured way based on the purification strategy of capture, intermediate purification, and polishing steps:

- 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

The typical protocols shown in this document (including recommended products) are good starting points when developing your methods. They have been tested by Cytiva's R&D team to maximize the purity/yield balance.

For the complete range of available products, visit cytiva.com

Abbreviations used

AC = affinity chromatography
AIEX = anion exchange chromatography
CIEX = cation exchange chromatography
HCP = host cell proteins
HIC = hydrophobic interaction chromatography
IEX = ion exchange chromatography
IMAC = immobilized metal ion affinity chromatography
PD = process development
SEC = size exclusion chromatography

Check list before you start

1 Define the level of purity that you need

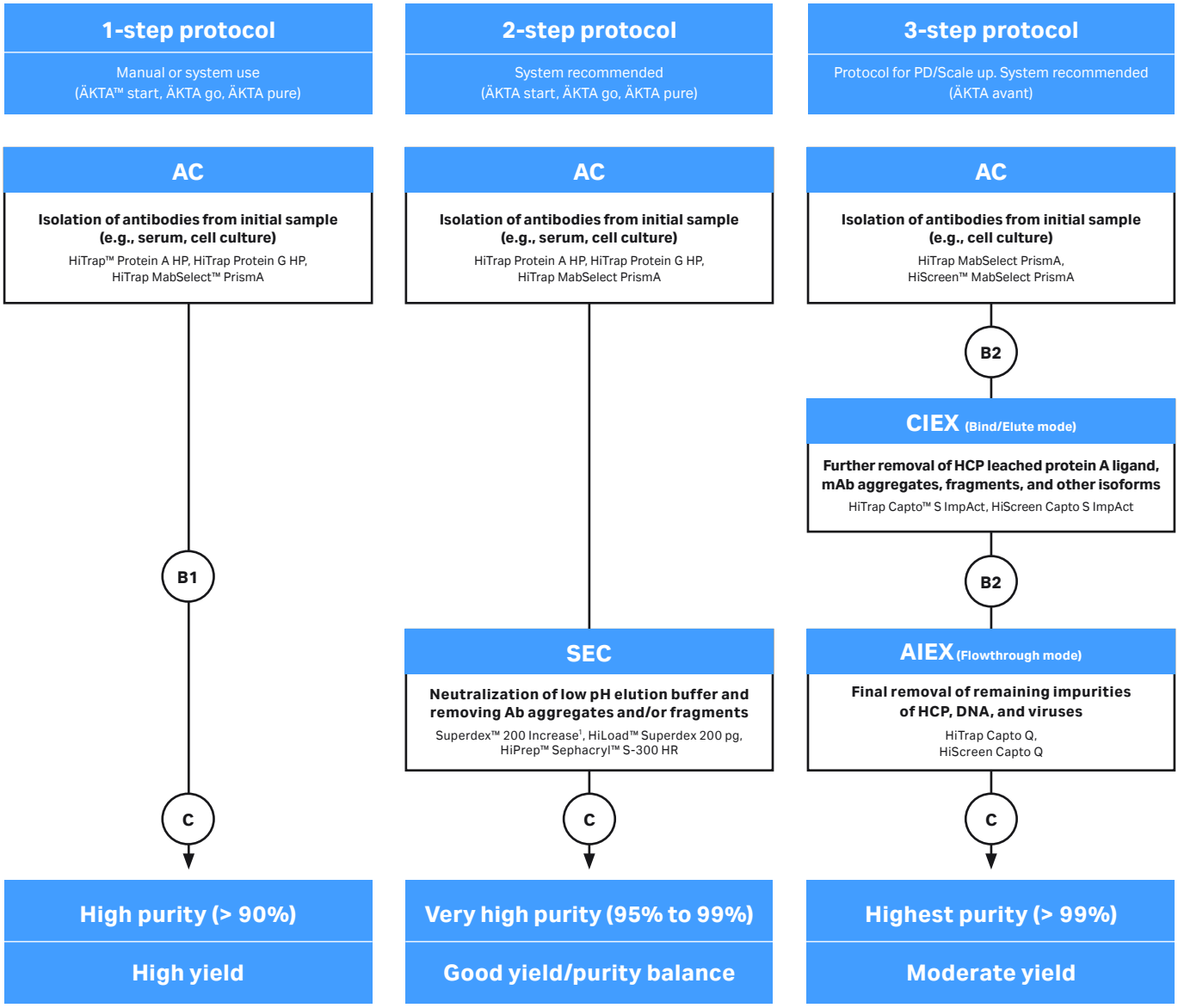
Typical applications	Purity level
Mass spectrometry **Antigen for immunization**	Moderate to high, 80% to 90%
Functional studies **Structural studies**	Very high, 95% to 99%
Structural studies **Therapeutic proteins**	Highest > 99%

2 Make sure that you have developed analytical assays to follow the progress of the purification.

3 Combine techniques in a logical way with minimized number of steps to obtain the expected purity/yield balance. The higher protein purity you need, the more purification steps you will have to deploy in your workflow. However, note that additional chromatography steps will increase purity but decrease yield of active protein.

4 Prevent reduction of activity: work quickly, at 4°C, remove proteases early.

Antibody purification



Protein A or protein G for Ab purification?

Protein G: good first choice for general purpose capture of antibodies at laboratory scale.
Protein A/PrismA: commonly preferred when purifying human monoclonal antibodies or developing processes for manufacturing scale applications.

Purifying multiple types of antibodies from different sources or batches?

HiTrap MabSelect PrismA columns: efficient cleaning with 1.0 M NaOH makes reuse of columns possible with minimal risk of cross-contamination. Antibody recovery is unaffected by the NaOH cleaning.

[1] Superdex Increase columns are not for use with ÄKTA start. ÄKTA go, ÄKTA pure and ÄKTA avant are recommended.

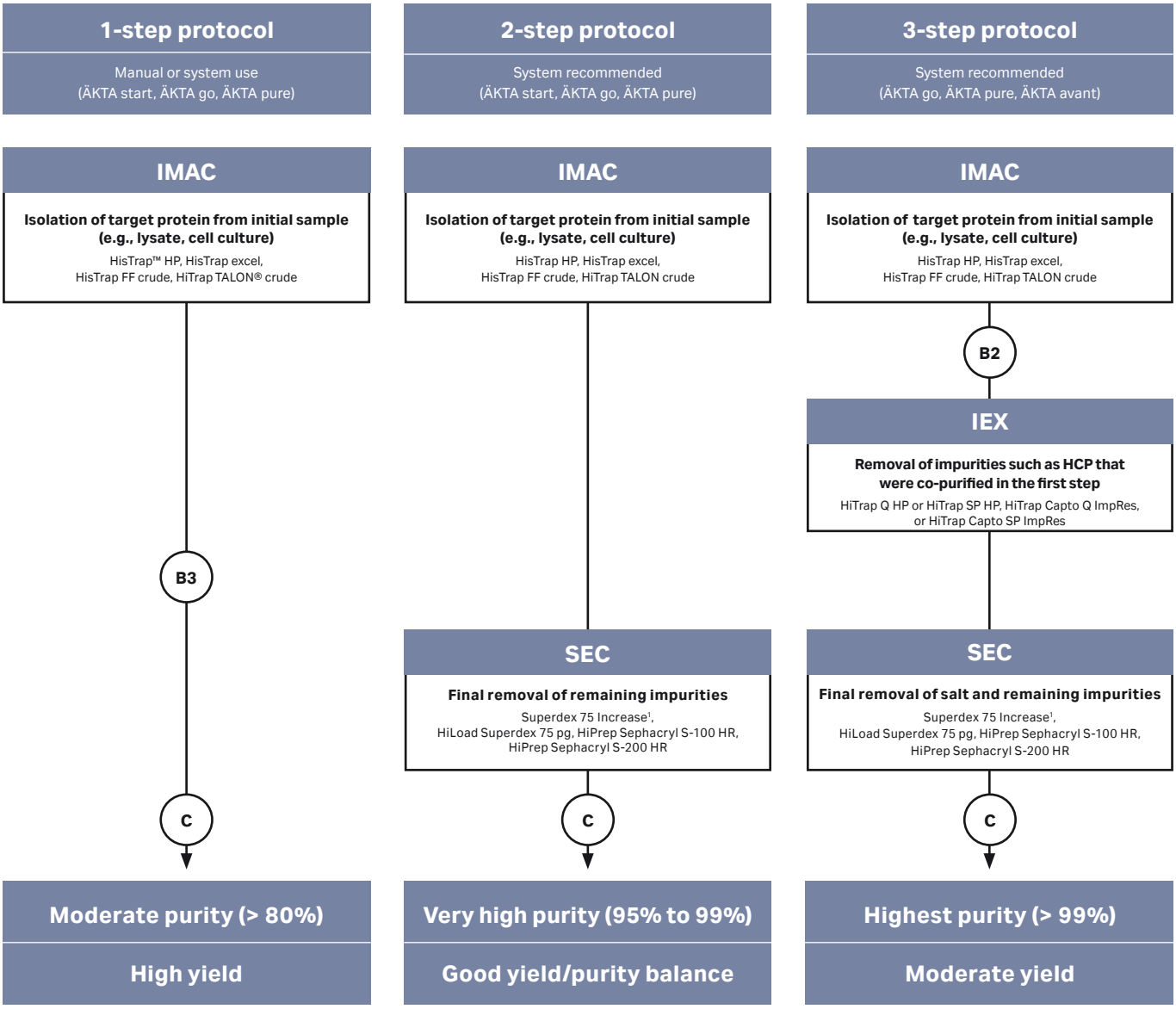
B1 **Buffer exchange to neutralize low pH Ab elution buffer.** (PD-10 Desalting, HiTrap Desalting columns)

B2 **Buffer exchange to prepare for IEX.** (HiTrap Desalting, HiPrep 26/10 Desalting columns)

C **Concentration for sample volume reduction. May also be performed before SEC.** (Vivaspin™ Sample Concentrators)

Steps in circles are optional and are applied if necessary.

His-tagged protein purification



Imidazole concentration: it is all about the purity/yield balance

- **To increase purity:** use a high imidazole concentration (> 20 mM) in the sample and binding buffer
- **To increase yield:** use no or a low imidazole concentration (0 to 5 mM) in the sample and binding buffer

Did the color of your nickel column change from green to a very light color?

Nickel stripping agents might be present in your sample. Choose HisTrap excel column, which contains a resin with strongly bound nickel ions.

[1] Superdex Increase columns are not for use with ÄKTA start. ÄKTA go, ÄKTA pure and ÄKTA avant are recommended.

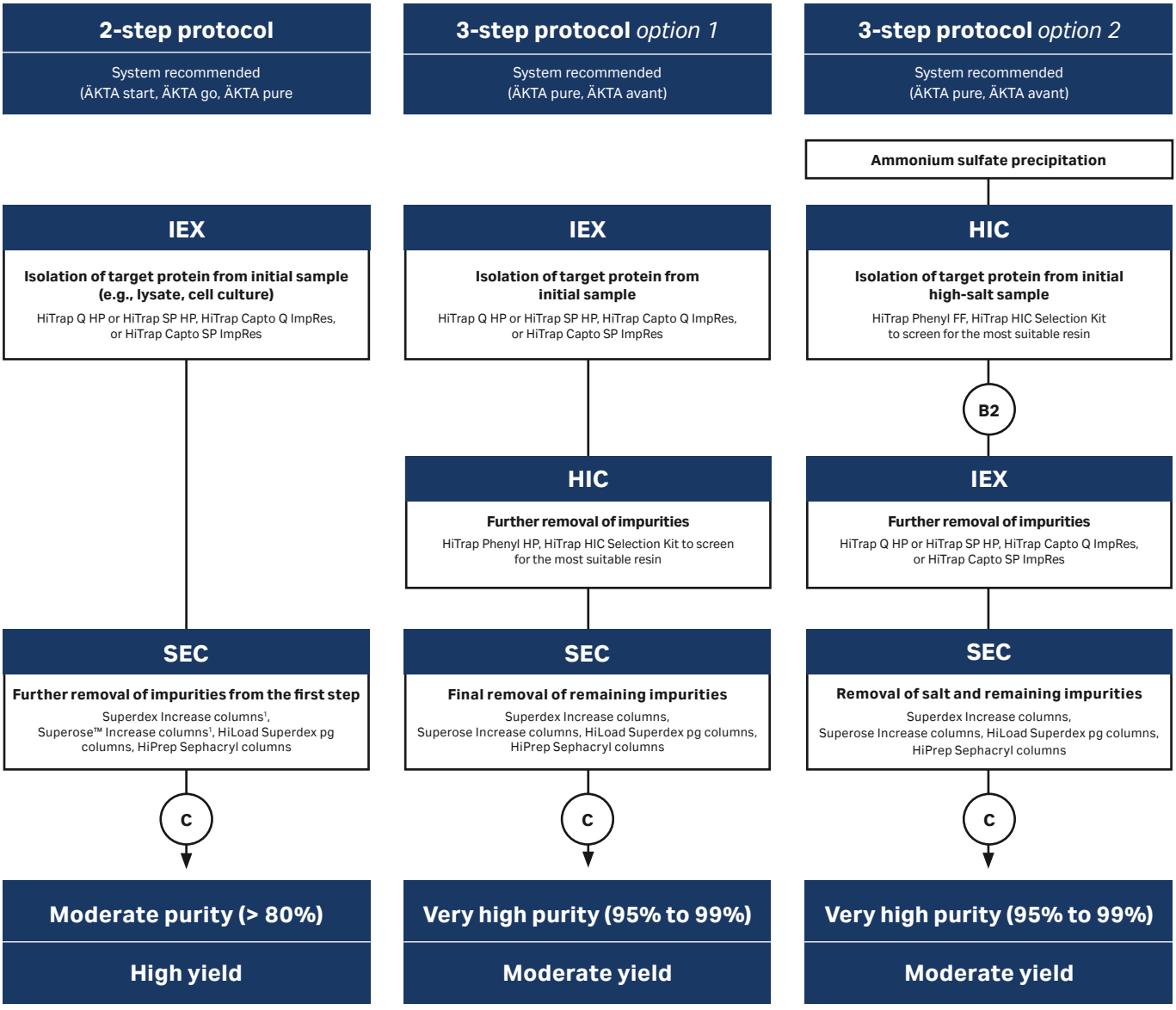
B2 **Buffer exchange to prepare for IEX.** (HiTrap Desalting, HiPrep 26/10 Desalting columns)

B3 **Buffer exchange to remove imidazole or salts.** (PD-10 Desalting, HiTrap Desalting columns)

C **Concentration for sample volume reduction. May also be performed before SEC.** (Vivaspin Sample Concentrators)

Steps in circles are optional and are applied if necessary.

Untagged protein purification



Why use ammonium sulfate precipitation in the 3-step protocol?

Ammonium sulfate is used for initial sample concentration and cleanup. It stabilizes proteins with no denaturation. The sample will contain a high salt concentration and may be applied directly to a HIC column with little or no additional preparation.

What about combining IEX techniques?

IEX is a method that offers different selectivity by using either cation (CIEX) or anion (AIEX) exchangers. A purification protocol can thus be designed to include a combination of CIEX and AIEX. The order of the IEX columns is dependent on the isoelectric point (pI) of the target protein and which contaminants you want to remove. Consider using the first column for binding and concentration of the target protein and the second column for binding of remaining contaminants (target protein flows through).

[1] Superdex Increase columns are not for use with ÄKTA start. ÄKTA go, ÄKTA pure and ÄKTA avant are recommended.

B2 **Buffer exchange to prepare for IEX.** (HiTrap Desalting, HiPrep 26/10 Desalting columns)

C **Concentration for sample volume reduction. May also be performed before SEC.** (Vivaspin Sample Concentrators)

Steps in circles are optional and are applied if necessary.

Recommendation for ÄKTA system users

Use predefined methods for protocol guidance

1 Create method[1]

Select a predefined method based on application, in ***Method editor*** by selecting ***File: New method***.

2 Adapt method

Adapt method to your conditions in ***Phase properties*** tab by selecting:

- Column in ***Method settings*** phase
- Column position in ***Method settings*** phase
- Way of fractionating in ***Elution*** phase

3 Prepare ÄKTA

Set up ÄKTA system with column(s), sample, buffers, tubing, and components.

4 Run method

- Select the created method in ***System control*** in the ***Method navigator***
- Click the ***Run*** button to run your method

[1] Relevant for ÄKTA go, ÄKTA pure, and ÄKTA avant. For ÄKTA start, same steps apply but software terms are named differently.

Ordering information for recommended prepacked columns

Antibody Purification

	Technique	Product name	Product code
1-step protocol	AC	Antibody package, protein A*	29058805
		Antibody package, protein G†	29058806
	AC	HiTrap MabSelect PrismA, 1 × 1 mL	17549851
		HiTrap Protein A HP 1 × 1 mL	29048576
		HiTrap Protein G HP 1 × 1 mL	29048581
	B1	PD-10 Desalting columns	17085101
		HiTrap Desalting, 1 × 5 ml	29048684
2-step protocol	AC	HiTrap MabSelect PrismA, 1 × 1 mL	17549851
		HiTrap Protein A HP 1 × 1 mL	29048576
		HiTrap Protein G HP 1 × 1 mL	29048581
	SEC	HiPrep 16/60 Sephacryl S-300 HR	17116701
		HiLoad 16/600 Superdex 200 pg	28989335
		Superdex 200 Increase 10/300 GL	28990944
3-step protocol	AC	HiTrap MabSelect PrismA, 1 × 1 mL	17549851
		HiScreen MabSelect PrismA	17549815
	CIEX	HiTrap Capto S ImpAct 1 × 1 mL	29400459
		HiScreen Capto S ImpAct	17371747
	AIEX	HiTrap Capto Q 1 × 1 mL	29401107
		HiScreen Capto Q	28926978
	B2	HiTrap Desalting, 1 × 5 ml	29048684
		HiPrep 26/10 Desalting	17508701
All protocols	C	Vivaspin Sample Concentrators	See cytiva.com

* Antibody package, Protein A includes: HiTrap Protein A HP , 1 × 1 mL , HiTrap Desalting, 1 × 5 mL
† Antibody package, Protein G includes: HiTrap Protein G HP , 1 × 1 mL , HiTrap Desalting, 1 × 5 mL

His-Tagged Protein Purification

	Technique	Product name	Product code
1-step protocol	IMAC	Tagged package, His[‡]	29058803
	IMAC	HisTrap HP 1 × 1 mL	29051021
		HiTrap TALON crude 1 × 1 mL	29048565
		HisTrap excel 1 × 1 mL	29048586
		HisTrap FF crude 1 × 1 mL	29048631
	B3	PD-10 Desalting columns	17085101
		HiTrap Desalting, 1 × 5 ml	29048684
2-step protocol	IMAC	HisTrap HP 1 × 1 mL	29051021
		HiTrap TALON crude 1 × 1 mL	29048565
		HisTrap excel 1 × 1 mL	29048586
		HisTrap FF crude 1 × 1 mL	29048631
	SEC	Superdex 75 Increase 10/300 GL	29148721
		HiLoad 16/600 Superdex 75 pg	28989333
		HiPrep 16/60 Sephacryl S-100 HR	17116501
		HiPrep 16/60 Sephacryl S-200 HR	17116601
3-step protocol	IMAC	HisTrap HP 1 × 1 mL	29051021
		HiTrap TALON crude 1 × 1 mL	29048565
		HisTrap excel 1 × 1 mL	29048586
		HisTrap FF crude 1 × 1 mL	29048631
	IEX	HiTrap Q HP 1 × 1 mL	29051325
		HiTrap SP HP 1 × 1 mL	29051324
		HiTrap Capto Q ImpRes 1 × 1 mL	29400462
		HiTrap Capto SP ImpRes 1 × 1 mL	29400460
	SEC	Superdex 75 Increase 10/300 GL	29148721
		HiLoad 16/600 Superdex 75 pg	28989333
		HiPrep 16/60 Sephacryl S-100 HR	17116501
		HiPrep 16/60 Sephacryl S-200 HR	17116601
	B2	HiTrap Desalting, 1 × 5 ml	29048684
		HiPrep 26/10 Desalting	17508701
All protocols	C	Vivaspin Sample Concentrators	See cytiva.com

[‡] Tagged package, His includes: HisTrap HP, 1 × 1 mL, HiTrap TALON crude, 1 × 1 mL, HiTrap Desalting, 1 × 5 mL

Untagged Protein Purification

	Technique	Product name	Product code
2-step protocol	IEX + SEC	Untagged package[#]	29058807
	IEX	HiTrap Q HP 1 × 1 mL	29051325
		HiTrap SP HP 1 × 1 mL	29051324
		HiTrap Capto Q ImpRes 1 × 1 mL	29400462
		HiTrap Capto SP ImpRes 1 × 1 mL	29400460
	SEC	Choice of column is dependent on the molecular weight of the protein	See cytiva.com
3-step protocol option 1	IEX	HiTrap Q HP 1 × 1 mL	29051325
		HiTrap SP HP 1 × 1 mL	29051324
		HiTrap Capto Q ImpRes 1 × 1 mL	29400462
		HiTrap Capto SP ImpRes 1 × 1 mL	29400460
	HIC	HiTrap Phenyl HP 5 × 1 mL	17135101
		HiTrap HIC Selection Kit	28411007
	SEC	Choice of column is dependent on the molecular weight of the protein	See cytiva.com
3-step protocol option 2	HIC	HiTrap Phenyl FF (high sub) 5 × 1 mL	17135501
		HiTrap HIC Selection Kit	28411007
	IEX	HiTrap Q HP 1 × 1 mL	29051325
		HiTrap SP HP 1 × 1 mL	29051324
		HiTrap Capto Q ImpRes 1 × 1 mL	29400462
		HiTrap Capto SP ImpRes 1 × 1 mL	29400460
	SEC	Choice of column is dependent on the molecular weight of the protein	See cytiva.com
	B2	HiTrap Desalting, 1 × 5 ml	29048684
		HiPrep 26/10 Desalting	17508701
All protocols	C	Vivaspin Sample Concentrators	See cytiva.com

[#] Untagged package includes: HITrap Q 1 × 1 mL, HiTrap SP HP 1 × 1 mL, HiPrep 16/60 Sephacryl S-200 HR

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