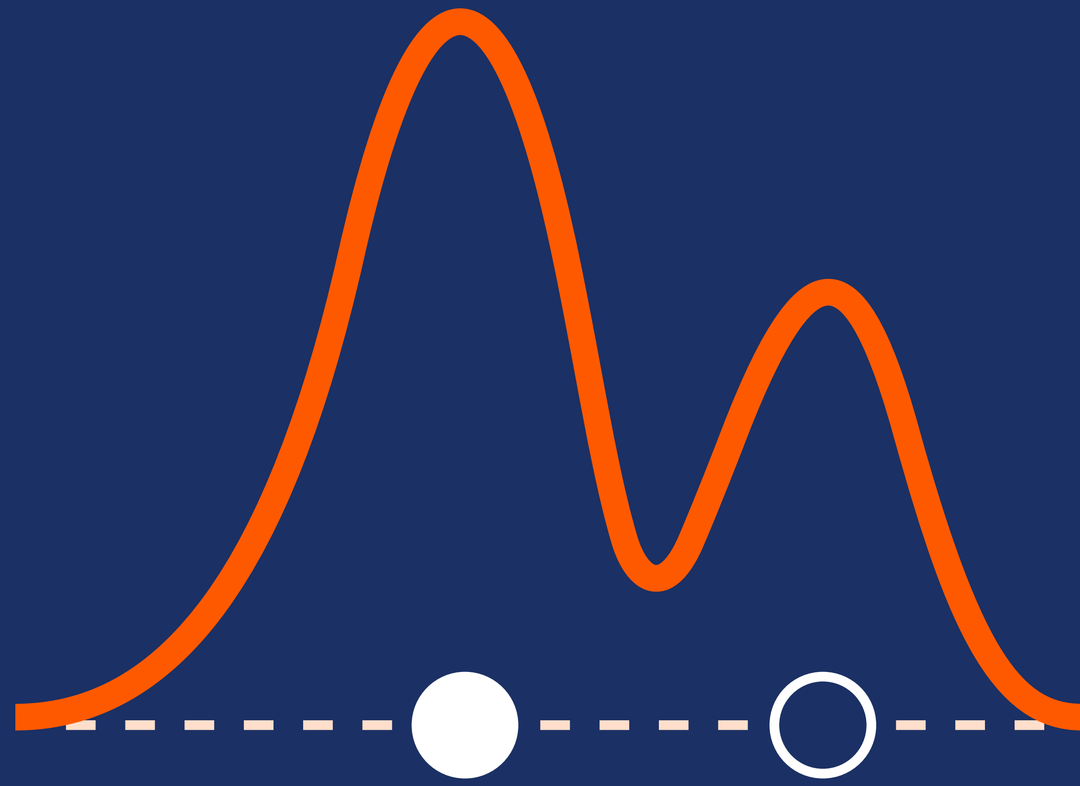
Histidine-tagged protein purification and detection

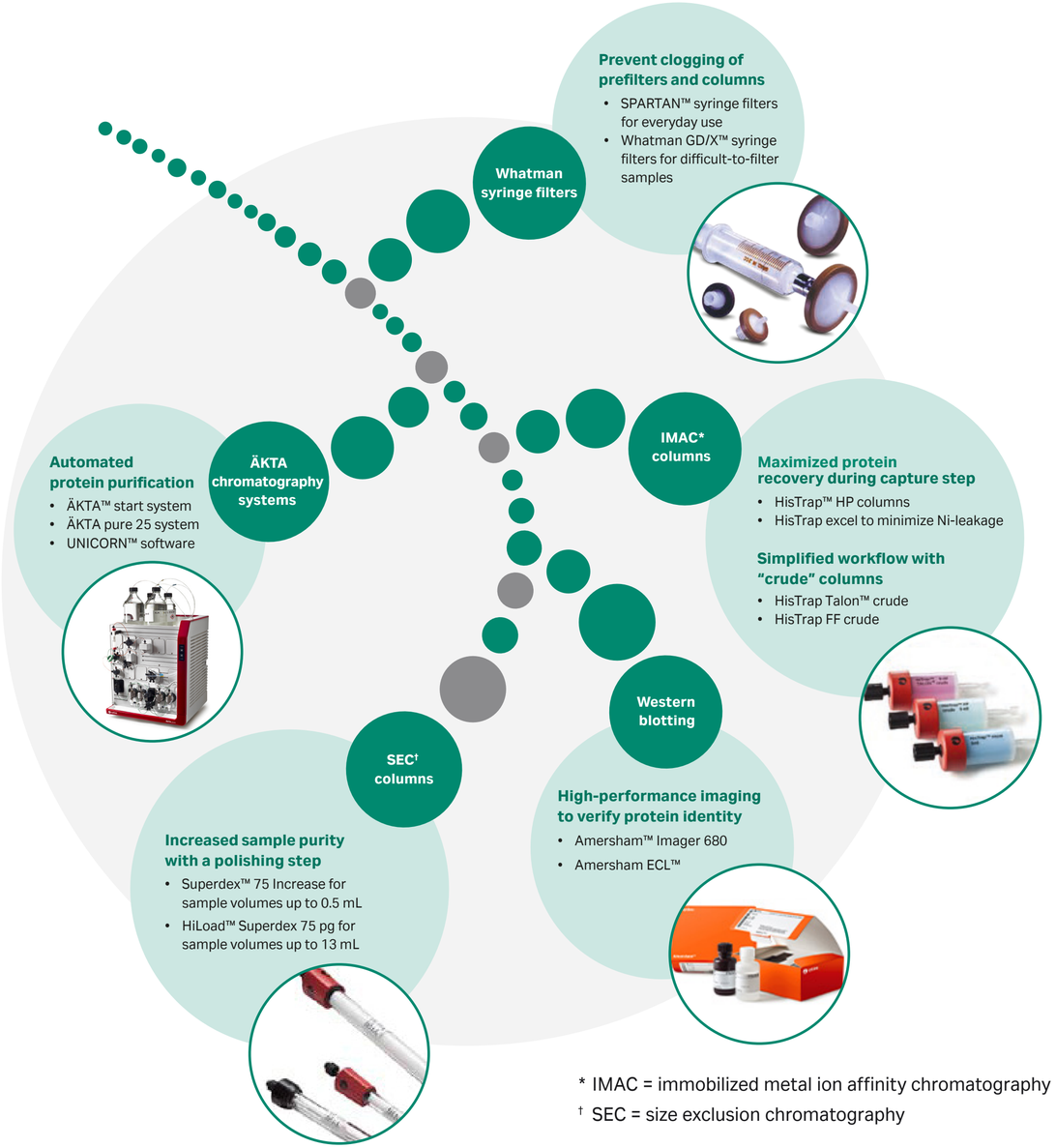
Prepare. Purify. Analyze.



cytiva

Introduction to histidine-tagged purification and analysis

Your histidine-tagged (his-tagged) purification and analysis workflow (Fig 1) includes sample preparation, filtration, purification, purity check and Western blotting for protein identification and/or quantitation. Each of the steps and products selected will influence the results in terms of recovery, purity and analytical quality but will also open opportunities to save time and money.



* IMAC = immobilized metal ion affinity chromatography

† SEC = size exclusion chromatography

Fig. 1. His-tagged protein purification workflow.

Use of IMAC for his-tagged protein purification

What is IMAC?

IMAC is based on the interaction of proteins with certain amino acid residues on their surface and divalent metal ions (e.g., Ni^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}) immobilized via a chelating ligand. The interaction is primarily between histidine and metal ions, but also, for example, tryptophan and cysteine. His-tagged proteins have extra high affinity in IMAC because of the multiple (6 to 10) histidine residues. These proteins are usually the strongest binder among all the proteins in a crude sample extract (e.g., a bacterial lysate), while other cellular proteins will not bind or will bind weakly.

What do his-tagged protein purification schemes look like?

On the right you will find some recommendations for purification of his-tagged proteins that can be considered when planning your experiment. The choice of techniques and combinations will depend on needed purity and yield for your protein of interest.

Please carefully define your objectives and consider that in general every added purification step (except for buffer exchange) will increase purity but decrease total protein recovery and yield. In Figure 2, we describe typical proven techniques combination for the purification of his-tagged proteins. If you consider a three-step purification protocol, the step from IMAC to IEX can be carried out directly if the buffer and pH conditions chosen in IMAC are compatible. This can typically be achieved if anion exchange columns/resins are selected. Please keep in mind that imidazole is a buffer substance as well, and that a sufficient washing phase should be applied after sample loading.

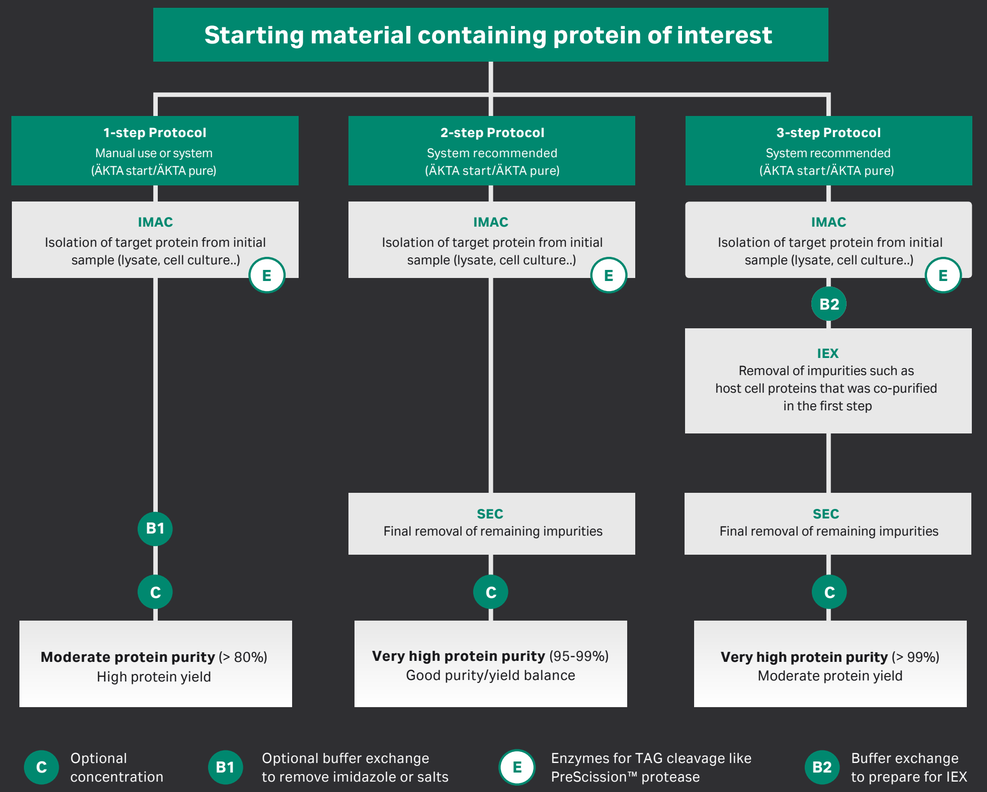


Fig. 2. Combining techniques for his-tagged purification with regards to yield and purity. The 2-step purification protocol is the recommended best choice. Steps in circles are optional and may only be applied on need base.

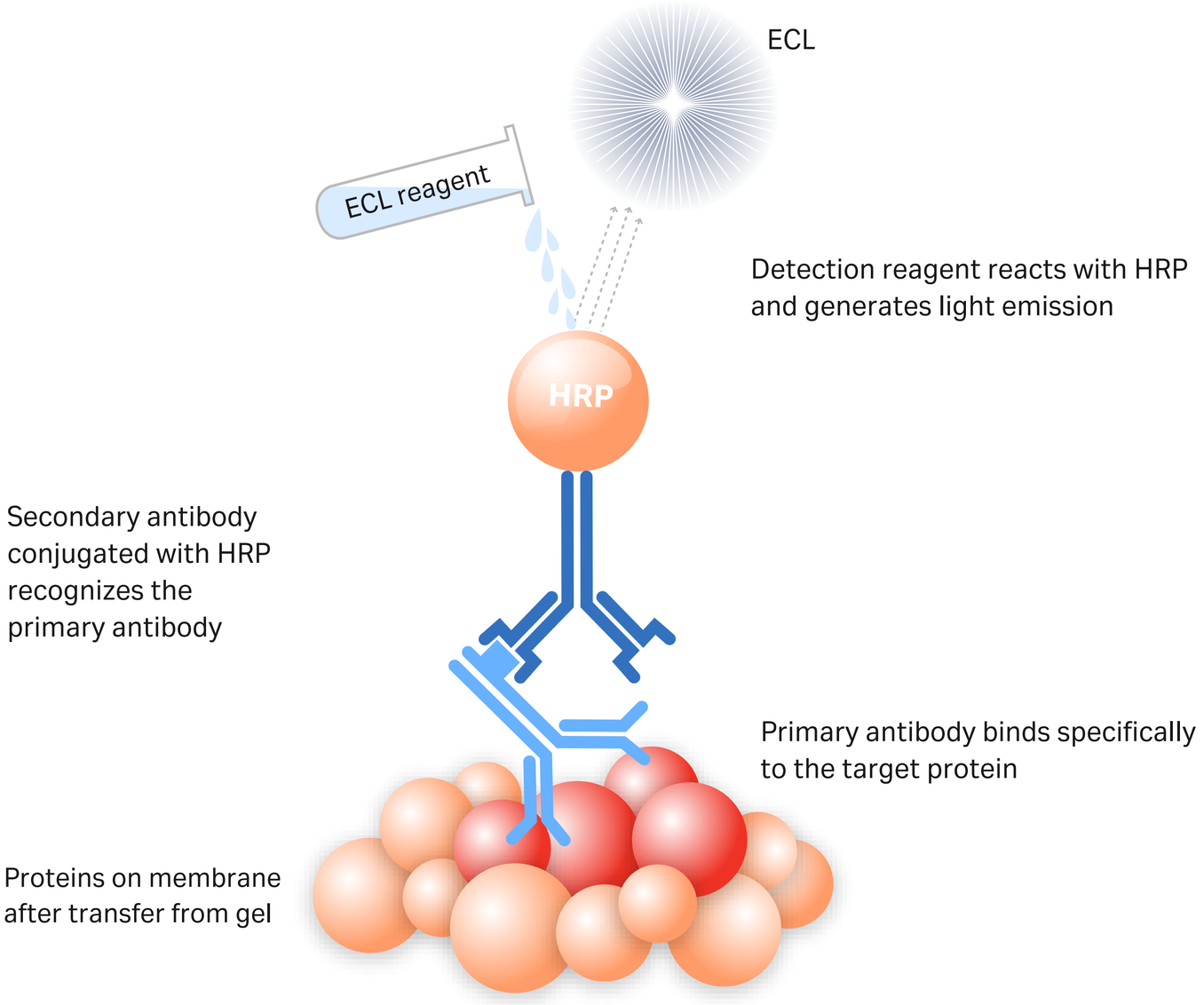
IMAC = immobilized metal ion affinity chromatography, IEX = ion exchange chromatography, SEC = size exclusion chromatography, TAG cleavage can be performed either after the IMAC step or while the tagged protein is still bound to the IMAC resin/column.

Use of Western blotting to verify protein identity and correct molecular weight

Western blotting, also known as immunoblotting, is a well-established and widely used technique for the detection and analysis of proteins. The method is based on building an antibody:protein complex via specific binding of antibodies to proteins immobilized on a membrane and detecting the bound antibody with one of several detection methods. The Western blotting method is one of the most commonly used methods in life science research. Western blotting has long been used for qualitative protein analysis to confirm protein presence and to approximately estimate protein amount. The development of highly sensitive detection reagents, however, together with advanced imaging techniques has made Western blotting a potential tool for quantitative protein analysis.

Chemiluminescence

In most contemporary ECL systems a luminol peroxide detection reagent is added to the membrane and reacts with the horseradish peroxidase enzyme (HRP) conjugated to the secondary antibody. HRP catalyzes the oxidation of luminol in a multistep reaction and is accompanied by the emission of low-intensity light at 428 nm, which can be measured with light-sensitive X-ray film or with a CCD imager.

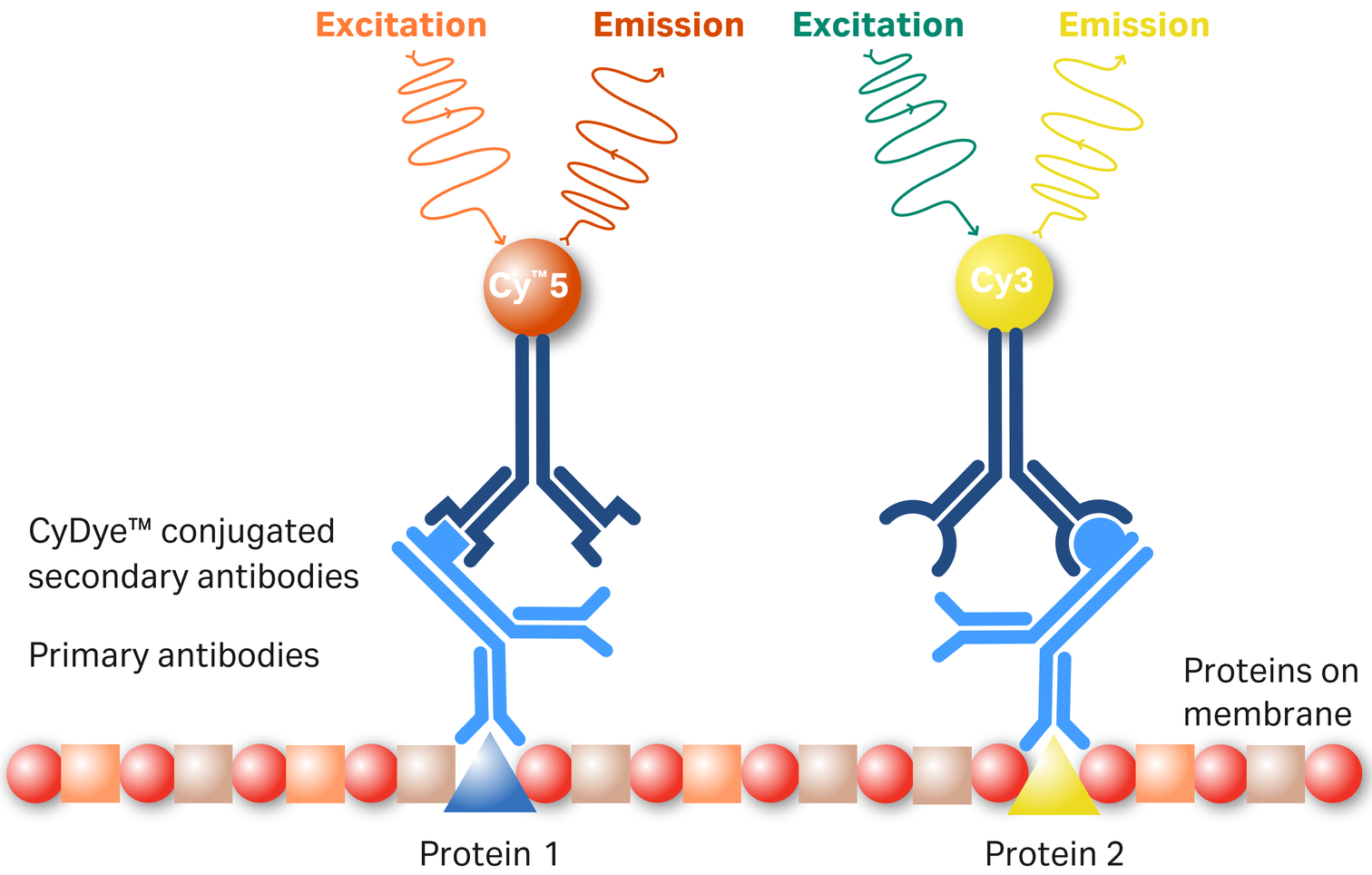


Fluorescence

Fluorescence detection is a direct method where the secondary antibody is conjugated to a fluorophore, thus avoiding the need for ancillary detection reagents.

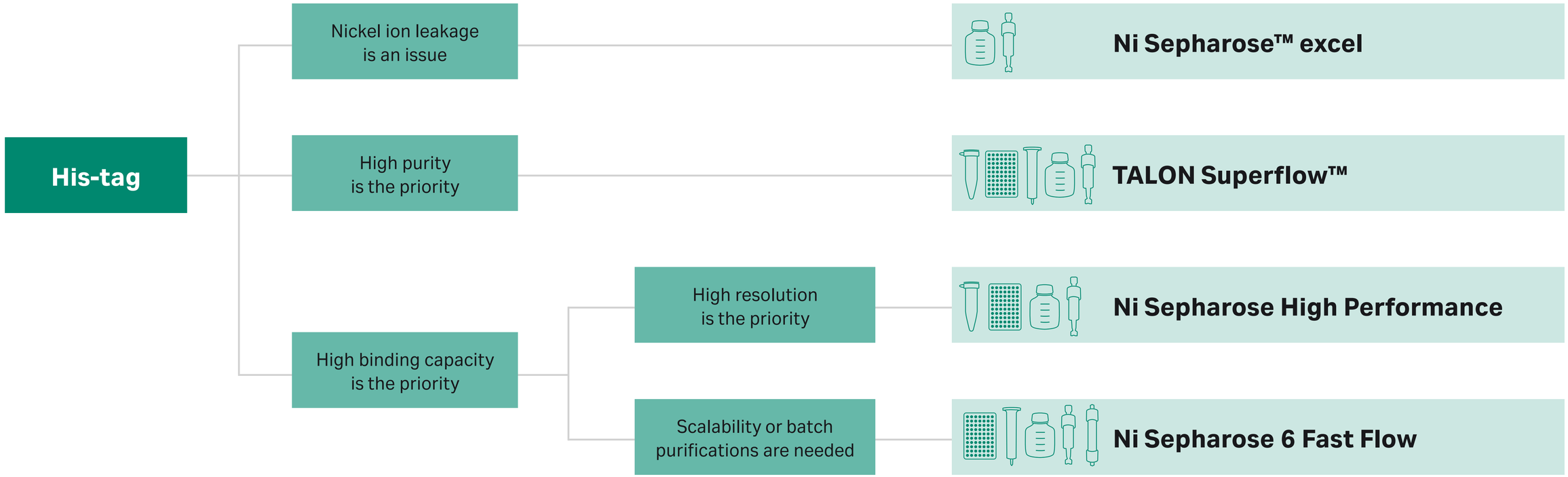
Fluorescence occurs when molecules called fluorophores absorb light. In their ground state, fluorophores do not emit light, but when subjected to light (excitation) their energy levels are raised to a brief but unstable excited state. As fluorophores return to their ground state, they release light at a lower energy, higher wavelength (emission) than that of the excitation light. Due to the stable signal, resulting in high reproducibility, fluorescence detection is the preferred method for quantitative Western blotting applications. In addition, if selected fluorescent dyes are spectrally resolvable (i.e., emit light of different wavelengths), they can be used as labels to allow multiplexing — the simultaneous detection of more than one target in a single sample.

Fluorescence detection is recommended for quantitation. This is because the signal stability and multiplexing capabilities result in reproducible data and normalization of target proteins in just one step.

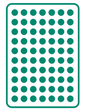


Guidance for chromatography resins and column selection

Select your IMAC resin



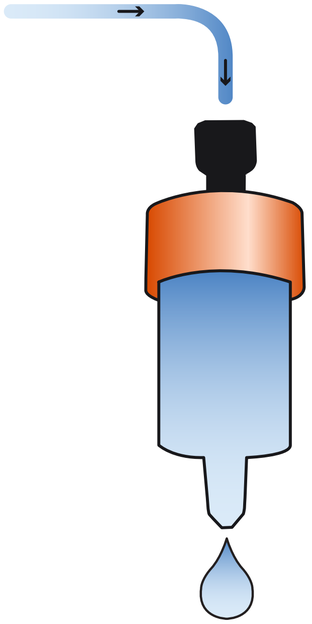
Select the format according to your needs

Type of purification	Manual purification			Manual or system purification		System purification
Symbol						
Format	Spin columns	96-well plates	Gravity flow columns	Bottles of chromatography resins	Small-column cartridges	Other columns
Format name	SpinTrap™	MultiTrap™	GraviTrap™, MiniTrap™, MidiTrap™, PD10	Lab pack	HiTrap (HisTrap)	HiScreen™, HiPrep™, HiLoad, RESOURCE™, Tricorn™, Precision
Use	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 purificaiton of proteins or sample desalting without the need for equipment	Batch purification and self-packing	Easy to use with a syringe, peristaltic pump, or a chromatography system	Larger scale or high-performance applications

Importance of DBC on yield and cost

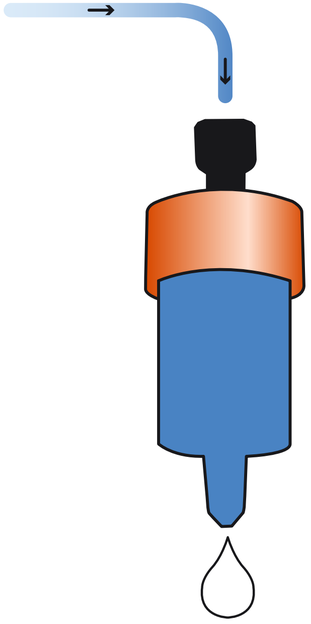
Dynamic binding capacity (DBC) describes the maximum amount of target protein that you can load onto your column without causing unnecessary loss measured under realistic experimental conditions (default flow-rate, real protein sample). In contrast to the total binding capacity (TBC) often shared by other vendors, DBC takes into account the risk of protein losses during purification with a column. At Cytiva, we always measure the dynamic binding capacity to show what you can really expect from the column.

A high dynamic binding capacity yields in more purified protein/mL resin in a prepacked column and, with that, reduces the cost/mg protein. It is worth evaluating the price of a product more based on DBC instead of package price.



Low DBC

Loss of target protein during sample application



High DBC

Minor loss of target protein during sample application

Ordering information

Whatman syringe filters

Membrane	Format	Description	Hold up volume	Pack size	Item
Polyethersulfone (PES)[1] with prefilter (for high particulate loaded samples)	25 mm, 0.2 µm	Whatman GD/X syringe filters, PES	Full housing: 1.4 mL, with air purge: 250 µL	150	6876-2502
	25 mm, 0.45 µm	Whatman GD/X syringe filters, PES		150	6876-2504
Regenerated cellulose (RC)[2] with prefilter (for high particulate loaded samples)	25 mm, 0.2 µm	Whatman GD/X syringe filters, RC	Full housing: 1.4 mL, with air purge: 250 µL	150	6887-2502
	25 mm, 0.45 µm	Whatman GD/X syringe filters, RC		150	6882-2504
Regenerated cellulose (RC)[2]	30 mm, 0.2 µm	SPARTAN syringe filters, RC		100	10463060
	30 mm, 0.2 µm	SPARTAN syringe filters, RC		500	10463062
	30 mm, 0.45 µm	SPARTAN syringe filters, RC		500	10463052

[1] PES — Hydrophilic membrane. Particularly suitable for filtration of serum, plasma and tissue culture solutions.

[2] RC — Hydrophilic membrane. Exhibits low levels of non-specific protein binding.

IMAC columns

IMAC is used for the isolation of target protein from initial sample (lysate, cell culture) using the affinity from a multi-histidine peptide sequence (typically 6 or more) to a charged metal ion.

Resin and dynamic binding capacity	Format	Description	Column volume	Pack size	Item
Ni Sepharose excel ~ 10 mg $(his)_6$-tagged protein/mL	HiTrap column	HisTrap excel 5 × 5 mL	5 mL	5 columns	17371206
		HisTrap excel 5 × 1 mL	1 mL	5 columns	17371205
		HisTrap excel 1 × 1 mL	1 mL	1 column	29048586
TALON Superflow ~ 20 mg $(his)_6$-tagged protein/mL	HiTrap column	HiTrap TALON crude 5 × 5 mL	5 mL	5 columns	28953767
		HiTrap TALON crude 5 × 1 mL	1 mL	5 columns	28953766
		HiTrap TALON crude 1 × 1 mL	1 mL	1 column	29048565
Ni Sepharose High Performance ~ 40 mg $(his)_6$-tagged protein/mL	HiTrap column	HisTrap HP 5 × 5 mL	5 mL	5 columns	17524802
		HisTrap HP 5 × 1 mL	1 mL	5 columns	17524701
		HisTrap HP 1 × 5 mL	5 mL	1 column	17524801
		HisTrap HP 1 × 1 mL	1 mL	1 column	29051021
		Tagged-Package His[1]	—	—	29058803
Ni Sepharose 6 Fast Flow ~ 40 mg $(his)_6$-tagged protein/mL	HiTrap column	HisTrap FF 5 × 5 mL	5 mL	5 columns	17525501
		HisTrap FF crude 5 × 5 mL	5 mL	5 columns	17528601
		HisTrap FF 5 × 1 mL	1 mL	5 columns	17531901
		HisTrap FF crude 5 × 1 mL	1 mL	5 columns	11000458
		HisTrap FF crude 1 × 1 mL	1 mL	1 column	29048631

[1] Starter pack for purification of His-tagged proteins contains 3 columns: HisTrap HP (1 mL), HiTrap TALON crude (1 mL) and HiTrap Desalting (5 mL).

Protein concentration units

A convenient way to reduce your sample volume after SEC (when a higher concentration of target protein is needed) is to concentrate it using membrane ultrafiltration.

Membrane	MWCO value	Description	Sample volume	Hold-up volume membrane	Pack size	Item
Polyethersulfone (PES)	10 000	VivaSpin™ 500	100 to 500 µL	< 5 µL	25	28932225
		VivaSpin 2	0.4 to 2 mL	< 10 µL	25	28932247
		VivaSpin 6	2 to 6 mL	< 10 µL	25	28932296
		VivaSpin 20	5 to 20 mL	< 20 µL	12	28932360
	30 000	VivaSpin 500	100 to 500 µL	< 5 µL	25	28932235
		VivaSpin 2	0.4 to 2 mL	< 10 µL	25	28932248
		VivaSpin 6	2 to 6 mL	< 10 µL	25	28932317
		VivaSpin 20	5 to 20 mL	< 20 µL	12	28932361

VivaSpin concentrators are designed for use with biological fluids and aqueous solutions. Compatible pH range is from pH 1 to 9. Further details on chemical compatibility can be found in the *VivaSpin data file*.

IEX columns

IEX is used as an intermediate step for removal of impurities such as host cell proteins or incomplete expressed and truncated target proteins that were co-purified in the first affinity step. Capto™ ImpRes resin is a new generation resin from Cytiva that delivers the same level of purity as Sepharose High Performance with higher pressure/flow properties.

Resin	Format	Description	Volume	Pack size	Item
Q Sepharose High Performance ~ 70 mg BSA/mL	HiTrap column	HiTrap Q HP 5 × 5 mL	5 mL	5 columns	17115401
		HiTrap Q HP 5 × 1 mL	1 mL	5 columns	17115301
	HiScreen column	HiScreen Q HP	4.7 mL	1 column	28950511
SP Sepharose High Performance ~ 55 mg ribonuclease A/mL	HiTrap column	HiTrap SP HP 5 × 5 mL	5 mL	5 columns	17115201
		HiTrap SP HP 5 × 1 mL	1 mL	5 columns	17115101
	HiScreen column	HiScreen SP HP	4.7 mL	1 column	28950515
Capto Q ImpRes > 95 mg BSA/mL	HiTrap column	HiTrap Capto Q ImpRes 5 × 5 mL	5 mL	5 columns	17547055
		HiTrap Capto Q ImpRes 5 × 1 mL	1 mL	5 columns	17547051
	HiScreen column	HiScreen Capto Q ImpRes	4.7 mL	1 column	17547015
Capto SP ImpRes > 70 mg lysozyme/mL	HiTrap column	HiTrap Capto SP ImpRes 5 × 5 mL	5 mL	5 columns	17546855
		HiTrap Capto SP ImpRes 5 × 1 mL	1 mL	5 columns	17546851
	HiScreen column	HiScreen Capto SP ImpRes	4.7 mL	1 column	17546815

SEC columns

Best used for final removal of remaining impurities and aggregates.

Resin	Format	Description	Sample volume	Column volume	Pack size	Item
Superdex 75 Increase[1] M_r: 3000 to 70 000 for globular proteins	Tricorn column Efficiency: > 43 000 N/m	Superdex 75 Increase 10/300 GL	< 500 µL	24 mL	1 column	29148721
	Tricorn column Efficiency: > 38 000 N/m	Superdex 75 Increase 5/150 GL	< 50 µL	3 mL	1 column	29148722
	Precision column Efficiency: > 43 000 N/m	Superdex 75 Increase 3.2/300	< 50 µL	2.4 mL	1 column	29148723
Superdex 75 prep grade M_r: 3000 to 70 000 for globular proteins	HiLoad column Efficiency: > 13 000 N/m	HiLoad 16/600 Superdex 75 pg	< 5 mL	120 mL	1 column	28989333
		HiLoad 26/600 Superdex 75 pg	< 13 mL	320 mL	1 column	28989334
Sephacryl™ S-100 High Resolution M_r: 1000 to 100 000 for globular proteins	HiPrep column Efficiency: > 5000 N/m	HiPrep 16/60 Sephacryl S-100 HR	< 5 mL	120 mL	1 column	17116501
		HiPrep 26/60 Sephacryl S-100 HR	< 13 mL	320 mL	1 column	17119401

[1] We recommend using Superdex 75 Increase columns on ÄKTA pure (ÄKTA start is not compatible with these columns).

Desalting columns for buffer exchange

In a 1-step protocol, buffer exchange is used to remove imidazole which sometimes disturbs applications downstream or negatively influences the stability. When using a 3-step protocol, buffer exchange of fractionated peaks is used to transfer the sample to the correct buffer conditions prior to the next IEX step.

Resin	Format	Description	Sample volume	Column volume	Pack size	Item
Sephadex™ G-25 Superfine Exclusion limit M_r 5000	HiTrap column	HiTrap Desalting, 5 × 5 mL[1]	0.1 to 1.5 mL[1]	5 mL	5 columns	17140801
		HiTrap Desalting, 1 × 5 mL[1]	0.1 to 1.5 mL[1]	3 mL	1 column	29048684
Sephadex G-25 Fine Exclusion limit M_r 5000	HiPrep column	HiPrep 26/10 Desalting[1]	≤ 15 mL[1]	53 mL	1 column	17508701
Sephadex G-25 Medium Exclusion limit M_r 5000	Gravity flow column	PD-10 Desalting Column[2]	1.0 to 2.5 mL	8.3 mL	30 columns	17085101
		PD MidiTrap G-25[3]	0.5 to 1 mL	3.5 mL	50 columns	28918008
		PD MiniTrap G-25[3]	0.1 to 0.5 mL	2.1 mL	50 columns	28918007
	Spin column	PD SpinTrap G-25	100 to 180 µL	600 µL	50 columns	28918004

[1] HiTrap and HiPrep: up to 3 columns can be easily connected in series to increase the sample volume if needed (up to 4.5 or 45 mL).

[2] PD-10 package: includes 1 × columns stand, 4 × PD-10 spin adaptors, 1 × Buffer tray, 30 × Bottom sleeve (PD-10 Buffer reservoir has to be ordered separately).

[3] MiniTrap and MidiTrap: 4 spin adaptors are included; additional spin adaptors are available for the different formats in a pack size of 10.

ÄKTA protein purification systems

ÄKTA lab-scale protein purification systems are designed for purification of biomolecules, providing speed, performance, and flexibility in research and process development. Within the range of ÄKTA lab-scale systems there are different alternatives focusing on ease of use and reliability addressing various research requirements.

ÄKTA start

ÄKTA pure 25

Way of working	ÄKTA start	ÄKTA pure 25
Chromatography techniques[1]	AC, DS, IEX, SEC	AC, DS, IEX, SEC, HIC, RPC
Simple, one-step desalting, buffer exchange	●	●
Automated and reproducible protein Purification including support for gradient elution	●	●
Method development and optimization using design of experiments (DoE)		○
Automatic multi-step purification		○
Column compatibility		
HisTrap, HiTrap and HiPrep SEC columns (16/60 and 26/60)	●	●
HiScreen, HiPrep Desalting and HiLoad SEC columns (16/600 and 26/600)		●
Superdex Increase columns (Tricorn and Precision)		●
General specifications		
Flow rate (mL/min)	0.5-5	0.001-25
Max. operating pressure/MPa)	0.5	20
Pump type	Peristaltic pump	Dual piston pump
UV monitor for real-time monitoring	280 nm LED	Single (280 nm LED) or triple wavelength (xenon flash, 190-700 nm)
Software[2] for system control and data handling	UNICORN start	UNICORN 6 or later
Fractionation	Frac 30[4]	F9-R[5], F9-C[6]
Ordering information		
Product code	29022094	29018224, 29018225, 29018226, 29018227, 29018228

Since the 1990s, ÄKTA systems have offered versatile and reliable protein purification. As a consequence of the renewal of the ÄKTA system platform, production of ÄKTAexplorer, AKTAprime plus, ÄKTApurifier, ÄKTAFPLC and ÄKTA micro has been discontinued. To improve your protein purification output we recommend upgrading to ÄKTA start, ÄKTA pure or ÄKTA avant. Please contact your Cytiva sales representative for further support or please visit cytiva.com/AKTAlabsystems for more details.

MPa = 10 bar, 145 psi; ● = included/compatible ○ = optional

1 AC = affinity chromatography, DS = desalting/buffer exchange, IEX = ion exchange chromatography, SEC = size exclusion chromatography, HIC = hydrophobic interaction chromatography, RPC = reversed phase chromatography.
2 A specific software version might be needed for the chosen system.
3 With PrimeView, you can monitor results and evaluate data but not create methods nor control the system.
4 Frac30 allows you to collect up to 30 fractions and supports four tube sizes, ranging from 1.5 to 15 mL. Fractions can be automatically collected in volumes ranging from 0.5 to 15 mL.
5 Add up to two (two round fraction collector, F9-R or one F9-R and one flexible fraction collector, F9-C). Up to 175 fractions per fraction collector, fraction volume: 0.1 to 50 mL, spillage-free mode: DropSync.
6 Up to 576 fractions, fraction volume: 0.1 to 250 mL, spillage-free mode: DropSync, accumulator, or automatic. The fraction collector is equipped with a variety of cassettes that can hold tubes (3, 8, 15, and 50 mL) as well as deep well plates (24-, 48-, and 96-well), for samples to be collected in the format needed. Six cassettes can be loaded into the fraction collector in any combination that fits the user's needs.

Detection and Western blotting

Detection method	Membrane and recommended detection	Description	Recommended use and signal duration	Quantity	Item
Chemiluminescence	PVDF (Amersham Hybond™ P) or nitrocellulose (Amersham Protran™) use best with: Amersham Hyperfilm™ ECL (X-ray film) or Amersham Imager 680, ImageQuant™ LAS 500	Amersham ECL start Western blotting detection reagent	For high abundance proteins; < 3 h	200 mL for 2000 cm^2 membrane	RPN3243
				400 mL for 4000 cm^2 membrane	RPN3244
		Amersham ECL Western blotting detection reagent	For high to medium abundance proteins; < 2 h	200 mL for 2000 cm^2 membrane	RPN2209
		Amersham ECL Prime Western blotting detection reagent	For medium to low abundance proteins; < 24 h	100 mL for 1000 cm^2 membrane	RPN2232
				300 mL for 3000 cm^2 membrane	RPN2236
		Amersham ECL Select™ Western blotting detection reagent	For low to very low abundance proteins; < 2 h	100 mL for 1000 cm^2 membrane	RPN2235
Fluorescence	Use best with: Amersham Typhoon™ series Amersham Imager 680 RGB	Amersham ECL Plex™ Goat-α-Rabbit IgG-Cy5	For low to very low abundance proteins; > 3 months	For 1000 cm^2 membrane	PA45011
		Amersham ECL Plex Goat-α-Mouse IgG-Cy5	For medium to very low abundance proteins; > 3 months	For 1000 cm^2 membrane	PA45009
		Amersham ECL Plex Goat-α-Rabbit IgG-Cy3	For medium to very low abundance proteins; > 3 months	For 1000 cm^2 membrane	28901106
		Amersham ECL Plex Goat-α-Mouse IgG-Cy3	For medium to very low abundance proteins; > 3 months	For 1000 cm^2 membrane	PA43009
Rainbow™ marker		Amersham ECL Full-Range Rainbow Molecular Weight Markers M_r 12 000 to 225 000 Ten separate proteins with six different colors	—	250 µL pack size sufficient for use with 50 minigels (10 × 8 cm) or 25 large gels (20 × 20 cm)	RPN800E
Western blotting membrane	For use with chemiluminescent and fluorescent detection methods for proteins of > M_r 20 000	Amersham Hybond P 0.45 PVDF Protein binding capacity: > 200 µg/cm^2	—	300 mm × 4 m 1 roll/pk	10600023
		Amersham Protran 0.45 NC Protein binding capacity: 115–125 µg/cm^2	—	300 mm × 4 m 1 roll/pk	10600002

For more product and pack size information plus additional products, please visit cytiva.com/westernblotting

Download our protein handbooks

Affinity chromatography handbooks

Affinity Chromatography Handbook, Vols. 1 to 3 present the most effective and most frequently used strategies for sample preparation and purification of proteins using affinity chromatography in the laboratory. The blend of general guidance and specific examples will be of enormous value to both the novice and the expert in developing a successful affinity purification strategy.

Affinity chromatography, vol. 1: Antibodies, Cytiva, 18103746 Edition AF (2016).

Affinity chromatography, vol. 2: Tagged proteins, Cytiva, 18114275 Edition AF (2016).

Affinity chromatography, vol. 3: Specific groups of biomolecules, Cytiva, 18102229 Edition AF (2016).

Further recommended handbooks

Strategies for protein purification, Cytiva, 28983331 Edition AA (2010).

Size exclusion chromatography: Principles and methods, Cytiva, 18102218 Edition AL (2014).

Western blotting: Principles and methods, Cytiva, 28999897 Edition AC (2014).

Imaging: Principles and methods, Cytiva, 29020301 Edition AA (2012).

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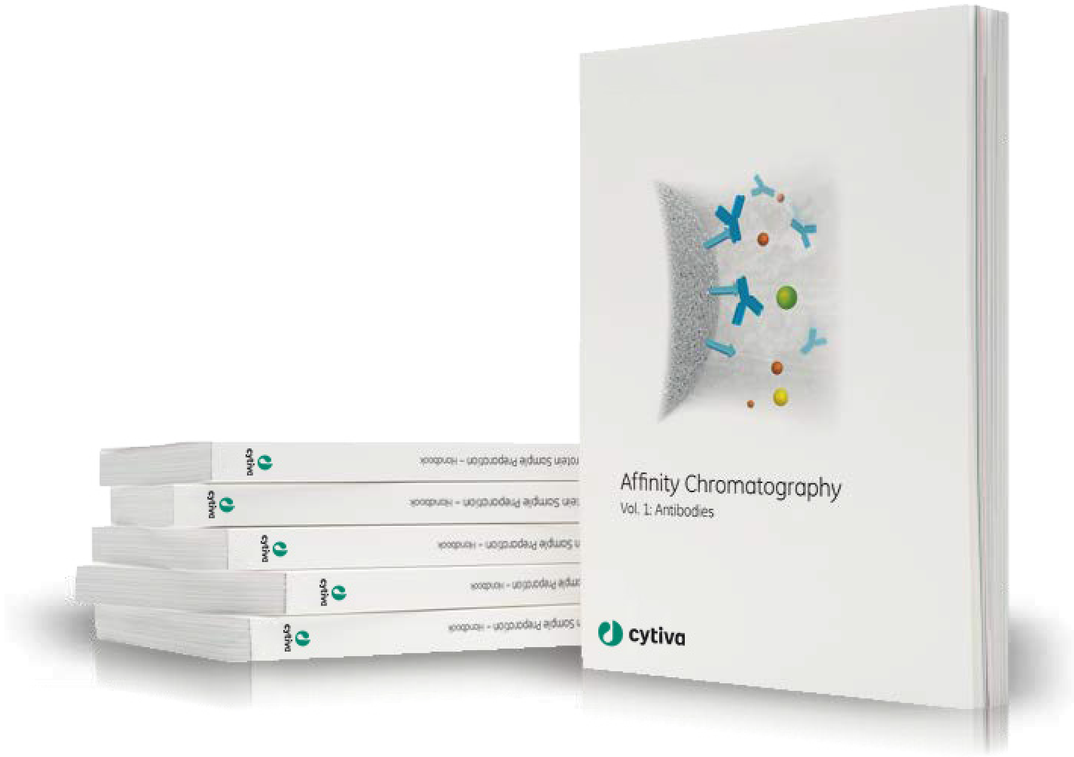

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Get further guidance on product selection

Selection guides for download

Selection guide: *Columns and resins for antibody purification and immunoprecipitation,* Cytiva, 28935197, Edition AC *(2016).*

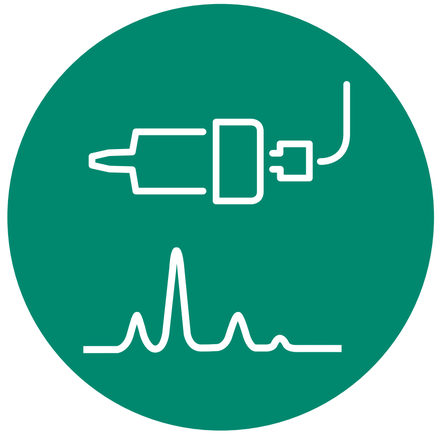
Selection guide: *Size exclusion chromatography columns and resin*, Cytiva, 18112419, Edition AK (2017).

Selection guide: *Your guide to chromatography resins Cytiva*, 29167217, AE (2017).

Poster: *Guide to modern BioProcess™ chromatography resins, Cytiva*, 29231394, Edition AB (2016).

Selection guide: *Prepacked chromatography columns for ÄKTA systems*, Cytiva, 28931778, Edition AL (2017).

Apps for use with a computer or mobile devices



Purify app — column and resin interactive selection tool

The Purify app simplifies the job of choosing the right chromatography resin and columns for your application. Based upon your answers to certain questions, the tool will guide you to a recommended product. From there, you can follow the link to the product page for more information.

Download the app on cytiva.com/purify

ÄKTA system accessories app

This guide will help you to quickly select the correct ÄKTA system accessories (tubing, frac racks, column holders, connectors and fittings). Pictures of different accessories will help you to identify the item you need. To support you in the ordering process there is an email function that enables you to email a list of selected items.

Download the app on cytiva.com/support/online-tools/chromatography/akta-accessories

Whatman filter selector

The Whatman filter selector from Cytiva business provides simple guide to choosing the correct Whatman filter and help take the guesswork out of filter selection.

Based on your answers to a few intuitive questions, the web-based interactive tools will help you select the right Whatman filter for your needs and provide technical data and related documents. No matter what area you work in, choosing the right filter for your application can save you time and simplify your processes.

Access the online tool on cytiva.com/whatmanselector

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For local office contact information, visit cytiva.com/contact

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