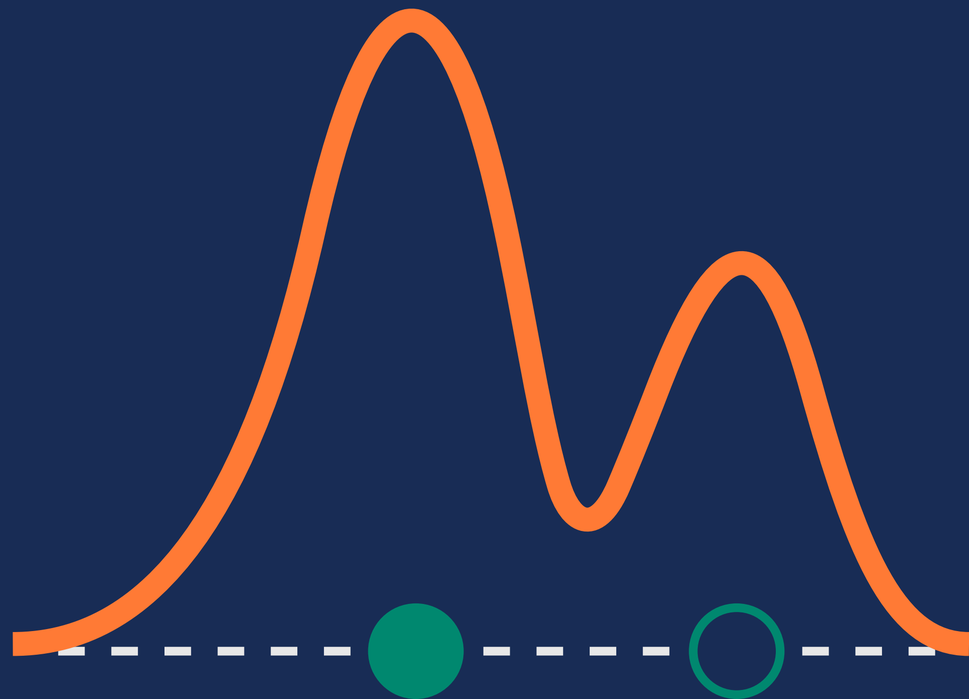
Antibody purification and immunoprecipitation

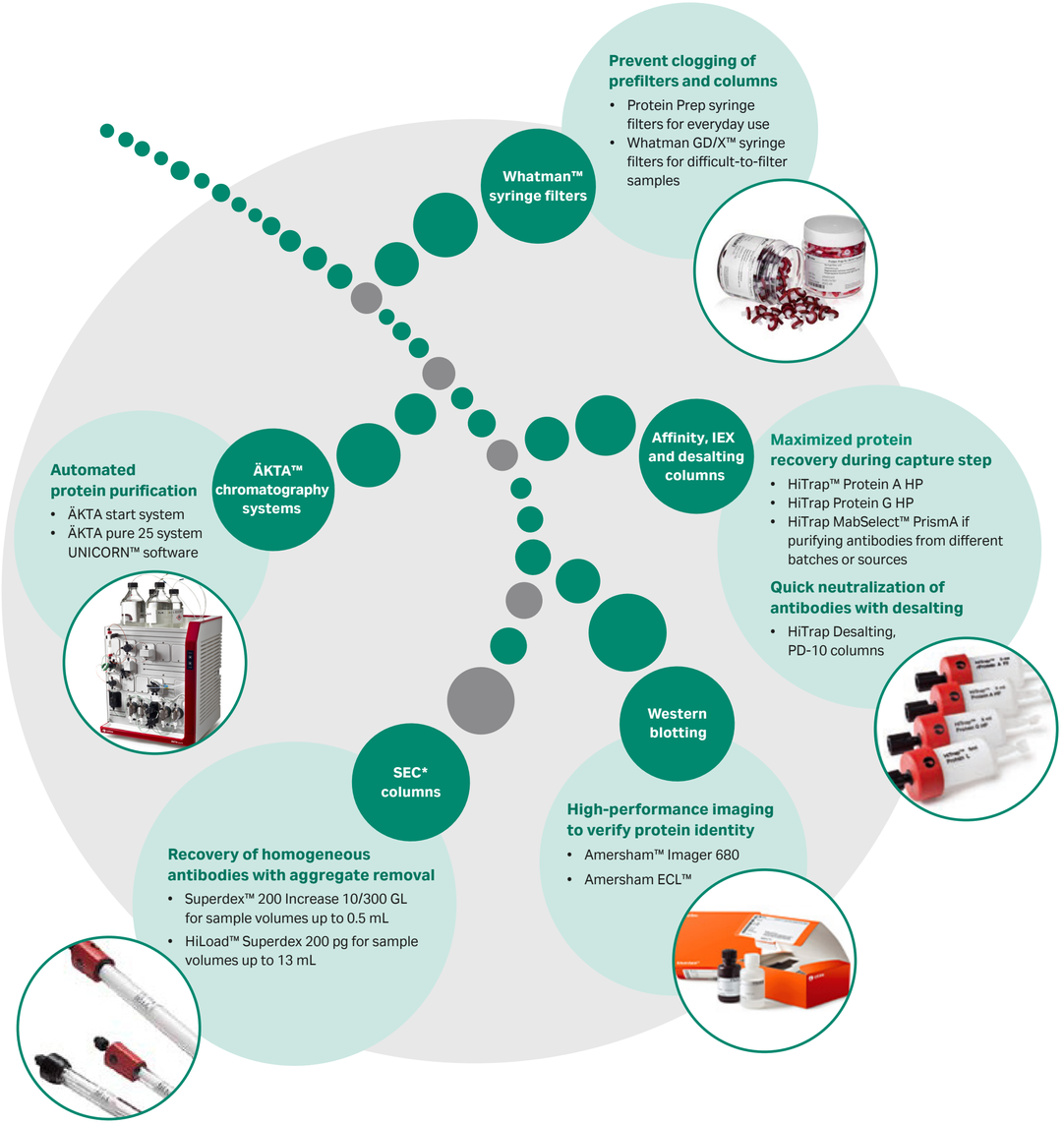
Prepare. Purify. Analyze.



cytiva

Introduction to antibody purification and analysis

Your antibody 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.



* SEC = size exclusion chromatography

Fig 1. Antibody purification and analysis workflow.

Use of affinity chromatography for antibody purification

How does antibody purification work?

Antibodies are members of a family of molecules, the immunoglobulins.

Polyclonal antibodies, monoclonal antibodies (mAb), and antibody fragments are usually purified by affinity chromatography. Resins containing an immobilized ligand (e.g., protein A, protein G, or protein L) are used to capture antibodies and antibody fragments (Fig 2).

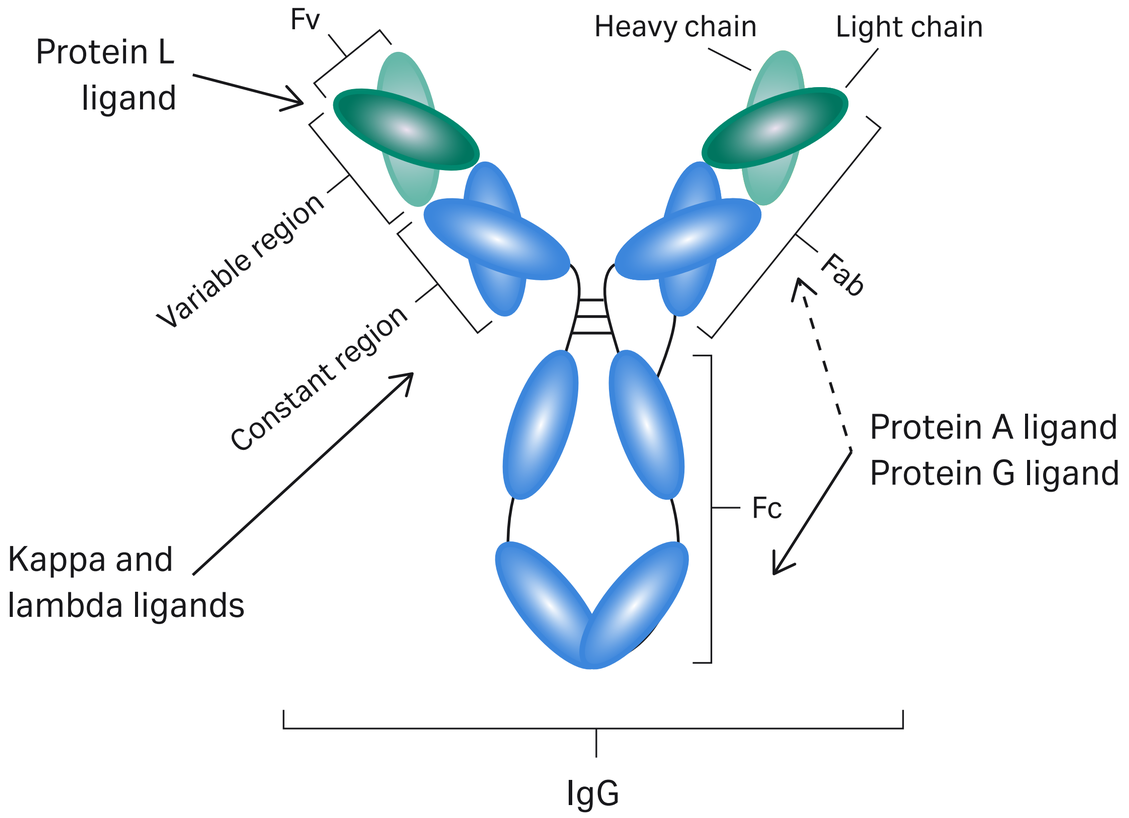


Fig 2. IgG, which is by far the most common immunoglobulin, is commonly purified with protein G and protein A, both of which have a strong affinity to the Fc region of IgG. Protein L has a strong affinity to the variable region of kappa light chains.

What do antibody purification schemes look like?

Antibody purification protocols typically are challenged by two factors. The first is specifically capturing as many antibodies as possible in the first step as well as controlling the degradation of the sample. The second is removing the remaining impurities and minimizing the aggregate content. To the right you will find suitable protocols to choose from (Fig 3).

The 3-step protocol considers upscaling or process development needs. SEC is not used as a final step to remove aggregates, fragment or other impurities due to the limitations of sample volume. Instead, a combination of IEX steps is used.

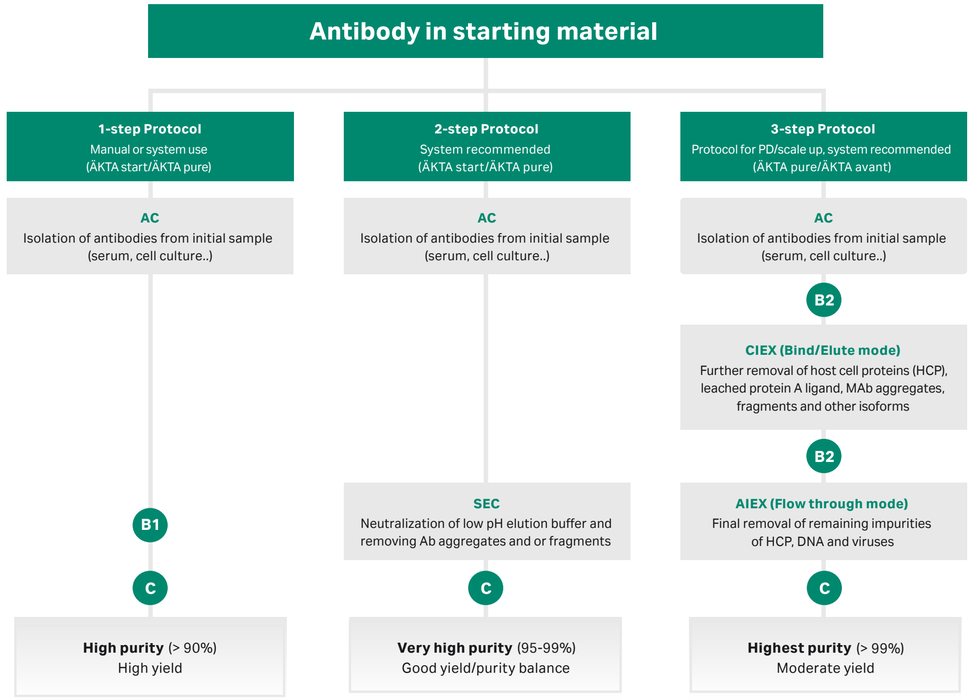


Fig. 3. Combining techniques for antibody purification with regards to yield and purity. The 2-step protocol is the recommended best choice for research use, whereas the 3-step protocol should be considered for scale-up or process development. Steps in circles are optional and may only be applied on an as required basis. AC = affinity chromatography, CIEX = cation ion exchange chromatography, AIEX = anion exchange chromatography, SEC = size exclusion chromatography.

C Optional concentration

B1 Buffer exchange to neutralize low pH elution buffer

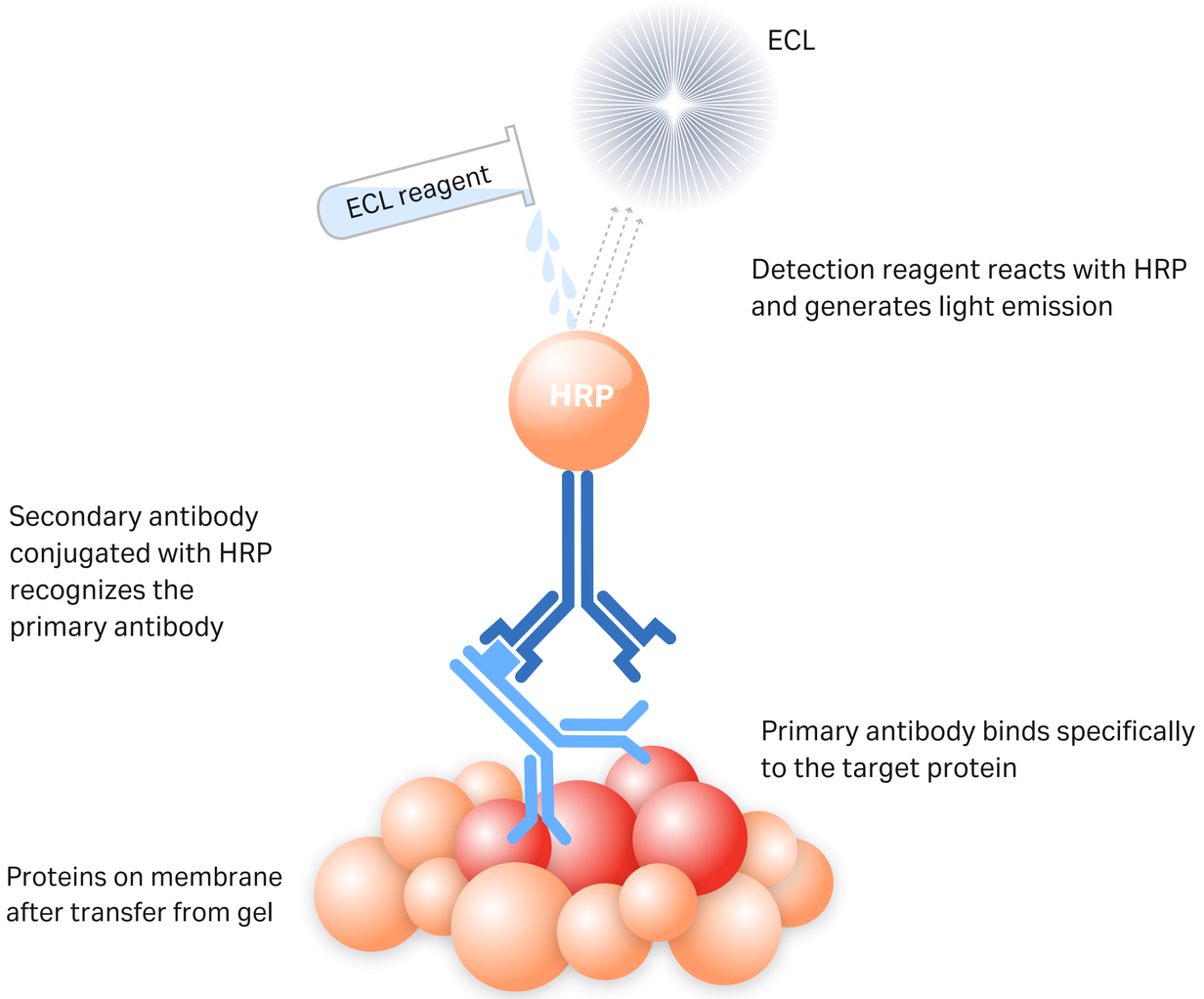
B2 Optional buffer exchange to prepare for IEX

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.

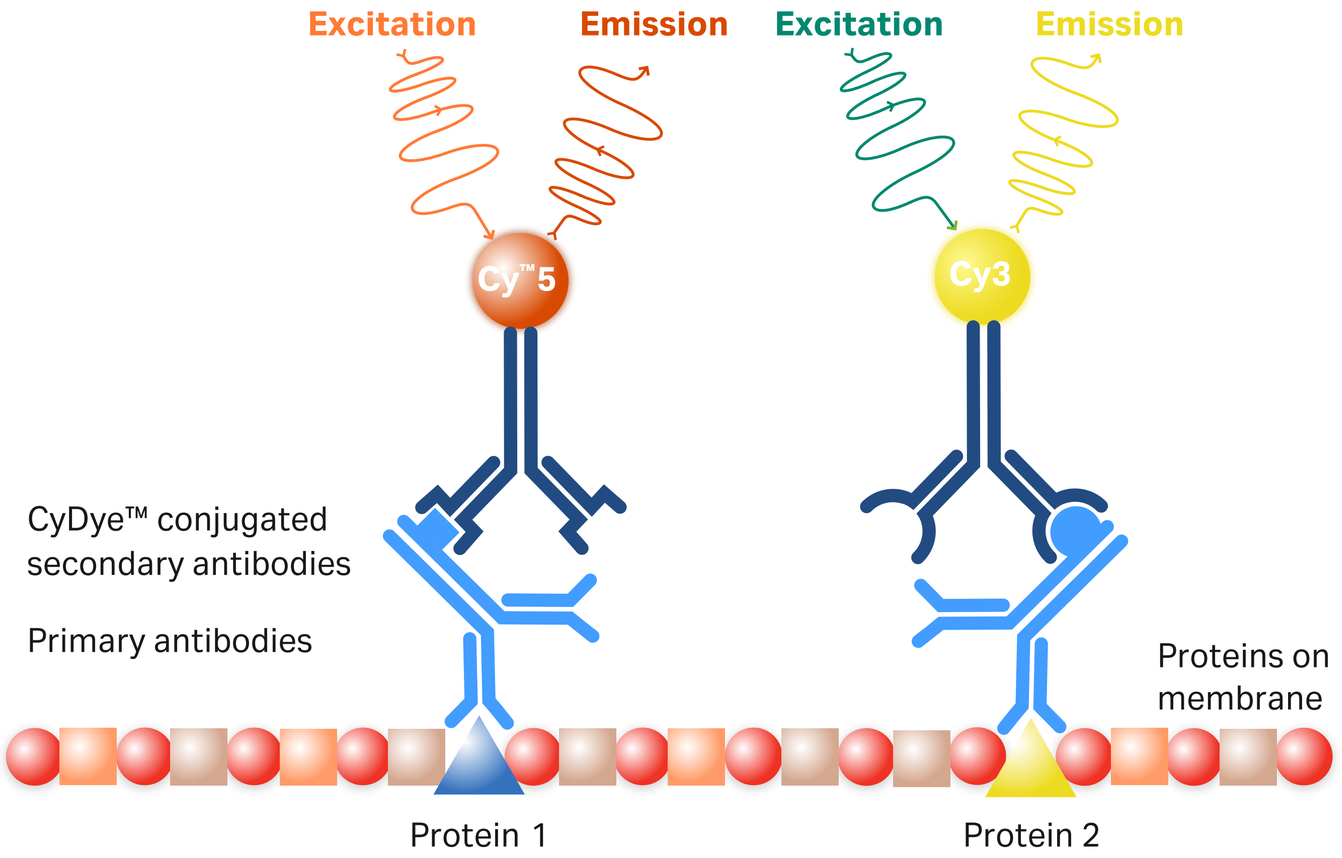


Fluorescence

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

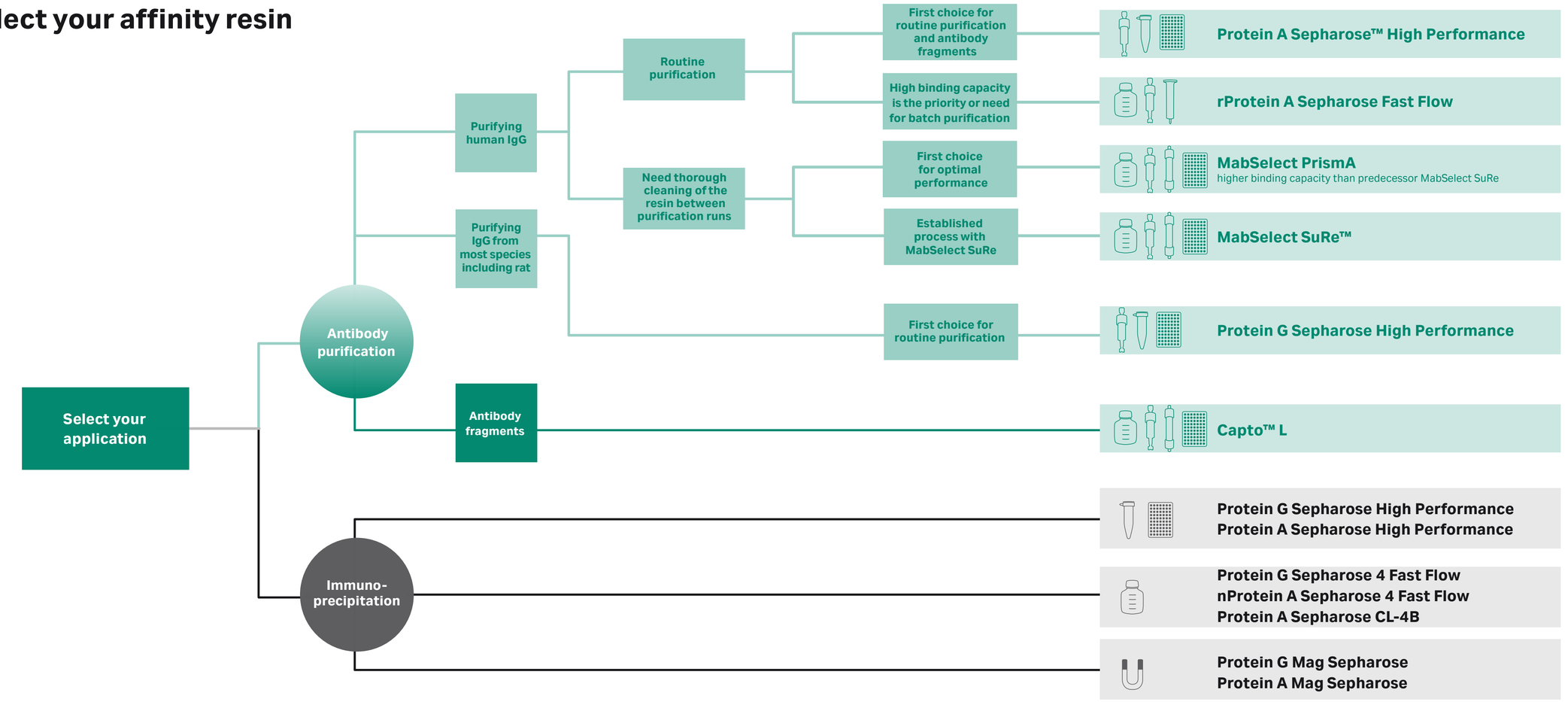
Fluorescence occurs when molecules called fluorophores absorb light. In their ground state, fluorophores do not emit light, but when subjected to light (excitation) their energy levels are raised to a brief but unstable excited state. As fluorophores return to their ground state, they release light at 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.

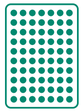


Guidance for chromatography resins and column selection

Select your affinity resin



Select the format according to your needs

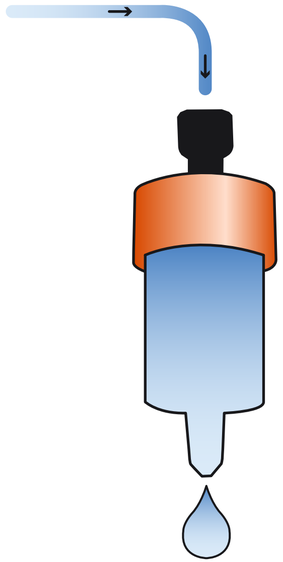
Type of purification	Manual purification			Manual or system purification			System purification
Symbol							
Format	Spin columns	96-well plates	Gravity flow columns	Bottles of chromatography resins	Magnetic beads	Small-column cartridges	Other columns
Format name	SpinTrap™	MultiTrap™	GraviTrap™, MiniTrap™, MidiTrap™, PD10	Lab pack	Mag Sepharose	HiTrap	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	Simplified enrichment of proteins, small-scale purification, and screening	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.

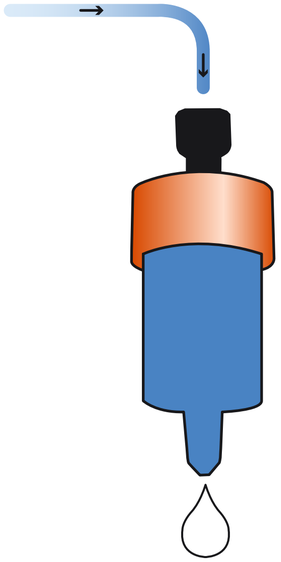
At Cytiva, we always measure the dynamic binding capacity to show what you can really expect from the column.

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	6887-2504
Regenerated cellulose (RC)[2, 3]	13 mm, 0.45 µm	Protein Prep syringe filter	Full housing: 135 µL, with air purge: < 10 µL	150	10463113
	13 mm, 0.2 µm	Protein Prep syringe filter		150	10463103
	30 mm, 0.45 µm	Protein Prep syringe filter		150	10463033
	30 mm, 0.2 µm	Protein Prep syringe filter		150	10463043

[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.

[3] Regenerated cellulose membrane shown to have average protein recovery > 97% and hold up volume < 10 µL (after air purge) tested with the 13 mm filter.

Affinity columns

Resin and dynamic binding capacity	Format	Description	Column volume	Pack size	Item
rProtein A Sepharose Fast Flow ~ 50 mg human IgG/mL	HiTrap column	HiTrap rProtein A FF 5 × 5 mL	5 mL	5 columns	17508002
		HiTrap rProtein A FF 5 × 1 mL	1 mL	5 columns	17507901
Protein A Sepharose High Performance ~ 20 mg IgG/mL	HiTrap column	HiTrap Protein A HP 5 × 5 mL	5 mL	5 columns	17040303
		HiTrap Protein A HP 5 × 1 mL	1 mL	5 columns	17040201
MabSelect SuRe ~ 35 mg human IgG/mL	HiTrap column	HiTrap MabSelect SuRe 5 × 5 mL	5 mL	5 columns	11003495
		HiTrap MabSelect SuRe 5 × 1 mL	1 mL	5 columns	11003493
MabSelect PrismA ~ 80 mg IgG/mL	HiTrap column	HiTrap MabSelect PrismA 5 × 5 mL	5 mL	5 columns	17549854
		HiTrap MabSelect PrismA 5 × 1 mL	1 mL	5 columns	17549852
Protein G Sepharose High Performance ~ 25 mg IgG/mL	HiTrap column	HiTrap Protein G HP 5 × 5 mL	5 mL	5 columns	17040503
		HiTrap Protein G HP 5 × 1 mL	1 mL	5 columns	17040401
Capto L 25 mg human Fab/mL	HiTrap column	HiTrap Protein L 5 × 5 mL	5 mL	5 columns	17547855
		HiTrap Protein L 5 × 1 mL	1 mL	5 columns	17547851

Protein concentration units

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

Resin	Format	Description	Volume	Pack size	Item
Capto Q ImpRes	HiTrap column	HiTrap Capto Q ImpRes 5 × 5 mL	5 mL	5 columns	17547055
> 95 mg BSA/mL		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	HiTrap column	HiTrap Capto SP ImpRes 5 × 5 mL	5 mL	5 columns	17546855
> 70 mg lysozyme/mL		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

Resin and fractionation range	Format	Description	Sample volume	Column volume	Pack size	Item
Superdex 200 Increase[1] M_r: 10 000 to 600 000 for globular proteins	Tricorn column efficiency: > 48 000 N/m	Superdex 200 Increase 10/300 GL	< 500 µL	24 mL	1 column	28990944
	Tricorn column efficiency: > 42 000 N/m	Superdex 200 Increase 5/150 GL	< 50 µL	3 mL	1 column	28990945
	Precision column efficiency: > 48 000 N/m	Superdex 200 Increase 3.2/300	< 50 µL	2.4 mL	1 column	28990946
Superdex 200 prep grade M_r: 10 000 to 600 000 for globular proteins	HiLoad column efficiency: > 13 000 N/m	HiLoad 16/600 Superdex 200 pg	< 5 mL	120 mL	1 column	28989335
		HiLoad 26/600 Superdex 200 pg	< 13 mL	320 mL	1 column	28989336
Sephacryl™ S-300 HR M_r: 10 000 to 1 500 000 for globular proteins	HiPrep column efficiency: > 5000 N/m	HiPrep 16/60 Sephacryl S-300 HR	< 5 mL	120 mL	1 column	17116701
		HiPrep 26/60 Sephacryl S-300 HR	< 13 mL	320 mL	1 column	17119601
Sephacryl S-200 HR M_r: 5000 to 250 000 for globular proteins	HiPrep column efficiency: > 5000 N/m	HiPrep 16/60 Sephacryl S-200 HR	< 5 mL	120 mL	1 column	17116601
		HiPrep 26/60 Sephacryl S-200 HR	< 13 mL	320 mL	1 column	17119501

[1] We recommend to use Superdex 200 Increase columns on ÄKTA pure (ÄKTA start is not compatible with these columns).

Desalting columns for buffer exchange

Resin and fractionation range	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]	0.1–1.5 mL[1]	5 mL	5 columns	17140801
		HiTrap Desalting, 1 × 5 mL[1]	0.1–1.5 mL[1]	5 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]	1.0–2.5 mL	8.3 mL	30 columns	17085101
		PD MidiTrap G-25[3]	0.5–1 mL	3.5 mL	50 columns	28918008
		PD MiniTrap G-25[3]	0.1–0.5 mL	2.1 mL	50 columns	28918007
	Spin column	PD SpinTrap G-25	100–180 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.

Immunoprecipitation columns and resins

Resin and dynamic binding capacity	Format	Description	Column volume	Pack size	Item
Protein G Sepharose High Performance ~ 25 mg IgG/mL	Spin column	Protein G HP SpinTrap	100 µL	16 columns	28903134
		Ab SpinTrap		50 columns	28408347
	96-well plate	Protein G HP MultiTrap	100 µL/well	4 plates	28903135
Protein A Sepharose High Performance ~ 20 mg IgG/mL	Spin column	Protein A HP SpinTrap	100 µL	16 columns	28903132
	96-well plate	Protein A HP MultiTrap	100 µL/well	4 plates	28903133
Protein G Sepharose 4 Fast Flow 20 mg IgG/mL	Resin in bulk	Protein G Separose 4 Fast Flow	5 mL	1 bottle	17061801
rProtein A Sepharose Fast Flow ~ 50 mg human IgG/mL	Resin in bulk	rProtein A Sepharose Fast Flow	5 mL	1 bottle	17127901
Protein A Sepharose CL-4B ~ 20 mg human IgG/mL	Resin in bulk	Protein A Sepharose CL-4B	1.5 g	1 bottle	17078001
Protein G Mag Sepharose ~ 13 mg human IgG/mL	Magnetic beads	Protein G Mag Sepharose	500 µL/vial	4 vials	28951379
Protein A Mag Sepharose ~ 8 mg human IgG/mL	Magnetic beads	Protein A Mag Sepharose	500 µL/vial	4 vials	28951378

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		
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, 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 per fraction, 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

Host cell protein (HCP) is a primary impurity and a critical quality attribute for biopharmaceuticals (biologics). HCP affects product quality, safety and efficacy. HCP ELISA is the gold standard of HCP detection and measurement, which requires polyclonal Antibodies (Ab) with broad reactivity against a wide range of potential HCPs.

2D differential in blot electrophoresis (2D-DIBE) combined with Western blotting is a powerful technology for separation and visualization of complex protein mixtures such as HCPs.










Sample preparations	Protein labeling	Protein separation	Protein transfer	Western blotting	Image acquisition	Image analysis
2-D Quant Kit	CyDye DIGE fluor Cy3/5	IPG buffer, DeStreak and DryStrip	Amersham Hybond™ PVDF and Protran™ NC	Blocking buffer	Amersham Typhoon™ RGB/5	Melanie™ 9 Coverage/DIGE
2-D Clean-Up Kit		IPGphor™ IEF unit	Whatman blotting paper	ECL Plex™ Cy3/5	IQ/OQ and service contract	
TriplePrep Kit		DALT gel	TE 77 Transfer unit			
		SE 600 unit				
		Mini VE unit				

Ordering information

Product	Description	Quantity	Item
2-D Quant Kit	Accurate determination of protein concentration in samples prepared for electrophoresis techniques	500 assays	80648356
2-D Clean-Up Kit	Designed to prepare samples for 2-D electrophoresis that otherwise produce poor 2-D results due to high conductivity, high levels of interfering substances or low concentration	50 samples	80648451
CyDye DIGE Cy3 minimal CyDye DIGE Cy5 minimal	Size- and charge-matched fluorescent dyes specifically designed for detecting protein abundance differences in 2-D Fluorescence Difference Gel Electrophoresis	10 nmol 10 nmol	25800861 25800862
PG Buffer pH 3–11 NL	Use with Immobiline DryStrip gels to improve protein solubility	1 mL	17600440
DeStreak Rehydration Solution	Improves reproducibility and quality of 2-D gels by preventing streaking. Eliminates extra spots caused by nonspecific oxidation of proteins	5 × 3 mL	17600319
Immobiline™ DryStrip pH 3–11 NL, 7 cm Immobiline DryStrip pH 3–11 NL, 13 cm	Broad pH 3–11 NL gradient Immobiline DryStrip gel for fast and efficient screening to gain a broad overview of total protein distribution	12 12	17600373 17600375
Amersham Hybond LFP PVDF 0.2 µm (254 mm × 4 m)	Much lower background fluorescence than other commercially available PVDF membranes, resulting in higher sensitivity. Protein binding capacity over 200 µg/cm^2	1 roll	10600022
Amersham Protran Premium NC 0.45 µm (300 mm × 4 m)	High binding capacity (162–180 µg IgG/cm^2)	1 roll	10600003
ECL Prime Blocking Reagent	For blocking nitrocellulose and PVDF membranes in Western blot applications, at least 20 miniblots	40 g	RPN418
ECL Plex Goat-Anti-Rabbit IgG Cy3 ECL Plex Goat-Anti-Rabbit IgG Cy5	Up to 1000 cm^2 membrane, for medium to very low abundance proteins > 3 months	150 µg 150 µg	25800862 PA45011

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 AM (2018).

Western blotting: Principles and methods, Cytiva, 28999897 Edition AD (2018).

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

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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 (2018).

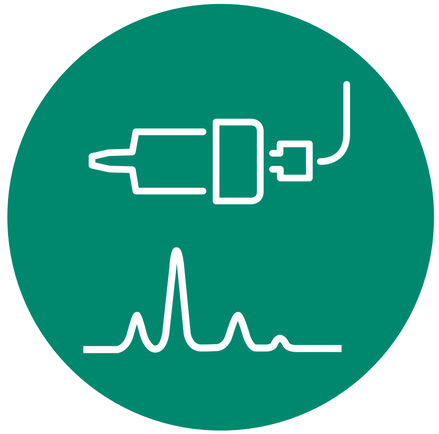
Selection guide: *Size exclusion chromatography columns and resins*, Cytiva, 18112419, Edition AJ (2017).

Selection guide: *Your guide to chromatography resins, Cytiva*, 29167217, AD (2018).

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

Selection guide: *Prepacked chromatography columns for ÄKTA systems*, Cytiva, 28931778, Edition AL (2018).

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 provides a simple guide to choosing the correct Whatman filter and helps 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

cytiva.com

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

CY18134-22Jan21-BR

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