Fisher Scientific

Get your antibody purification and immunoprecipitation right from the start

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Whatman™ syringe filters

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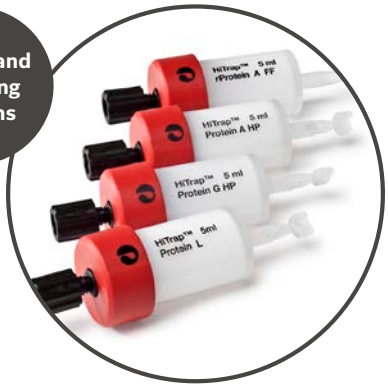


ÄKTA™ chromatography systems

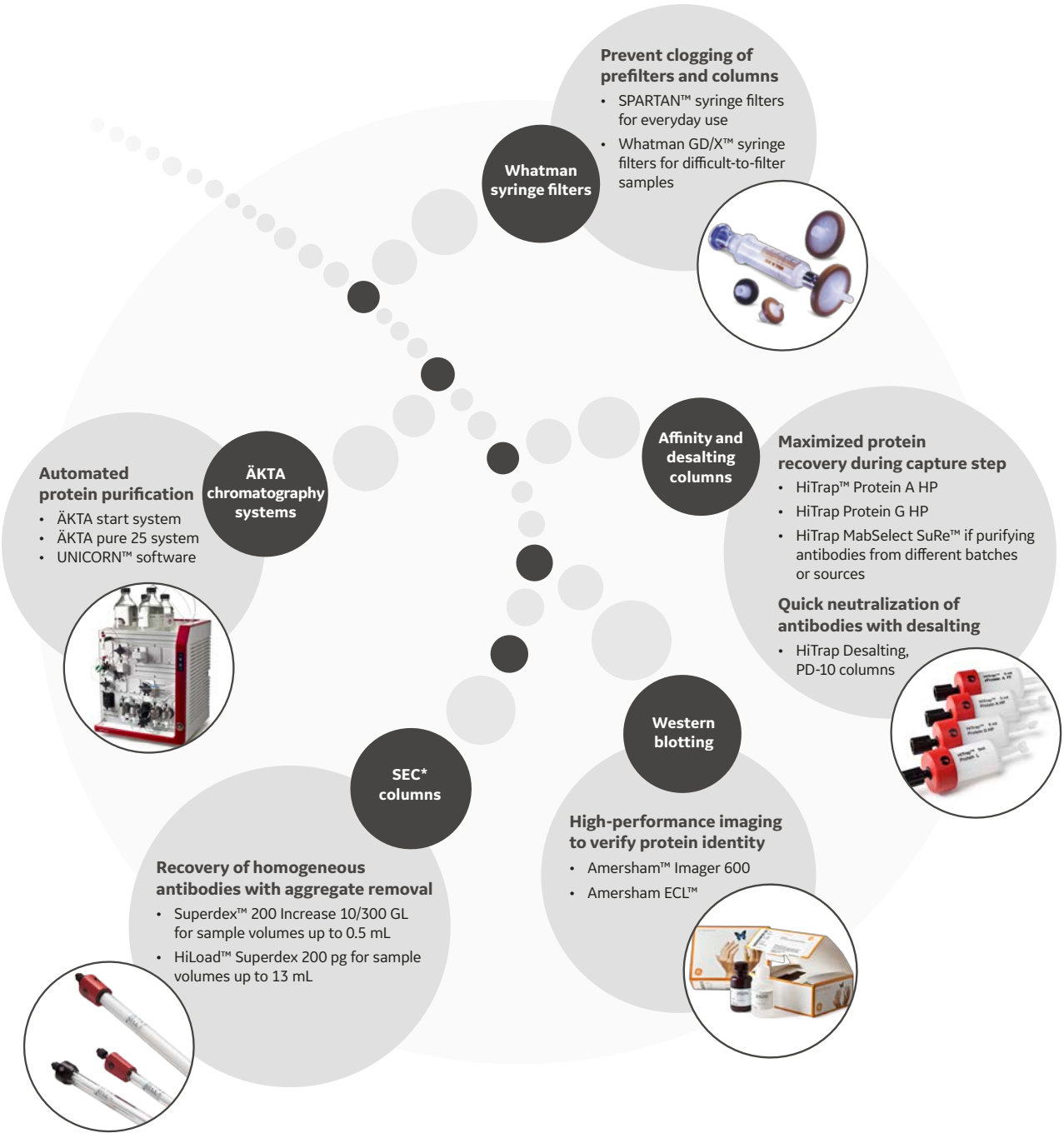
Western blotting

SEC columns

Distributor
GE Healthcare

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 also will 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 and immunoprecipitation

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).

Affinity purification offers high selectivity. Purity levels above 95% are often possible in just one step.

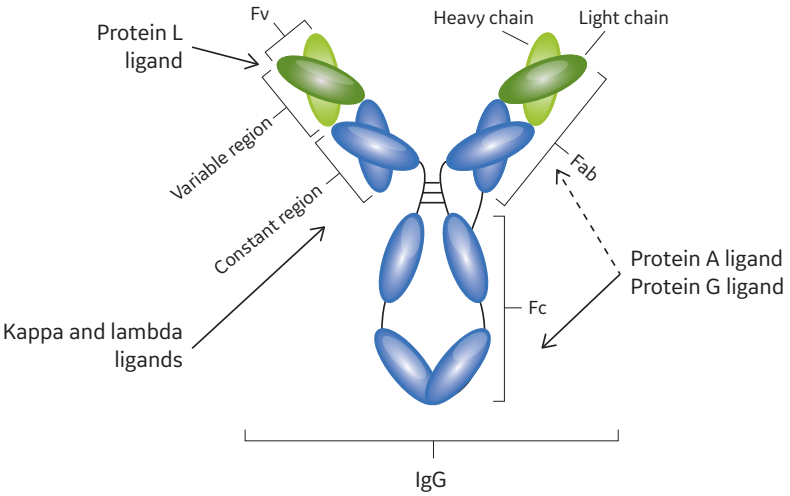


Fig 2. IgG, which is by far the most common immunoglobulin, is commonly purified with protein G and protein A, both of which have a strong affinity to the Fc region of IgG. Protein L has a strong affinity to the variable region of kappa light chains.

How does antibody purification work?

Antibody affinity chromatography is based on the high affinity and specificity to affinity ligands. The binding of an antibody to the ligand is reversible and the antibody is often eluted by lowering the pH (Fig 3).

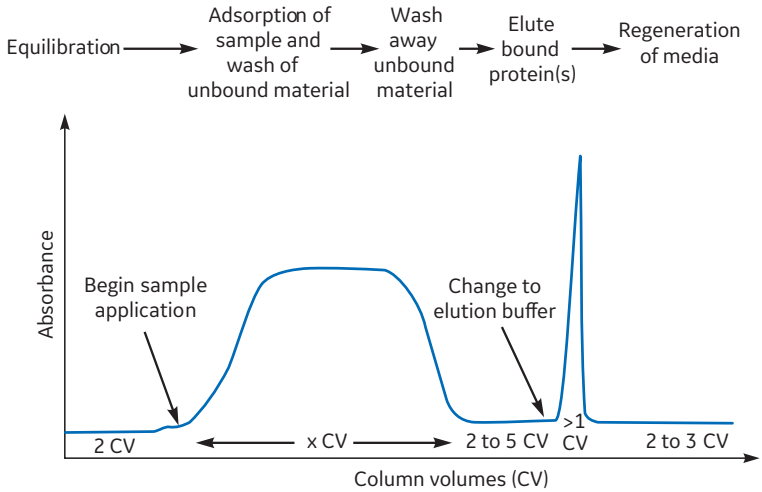


Fig 3. Typical affinity purification executed by a chromatography system.

What is immunoprecipitation?

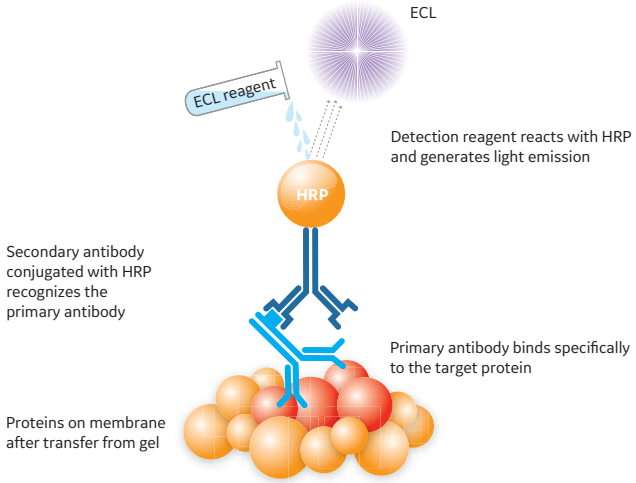
Immunoprecipitation (IP) is a highly specific and effective technique for analytical separations of target antigens from crude cell lysates. When combined with other techniques, such as SDS-PAGE and immunoblotting, IP can be used to analyze and quantitate your antigen.

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.

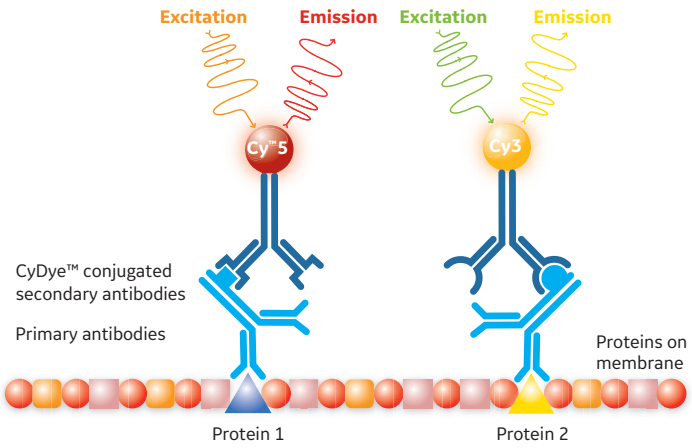


Fluorescence

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

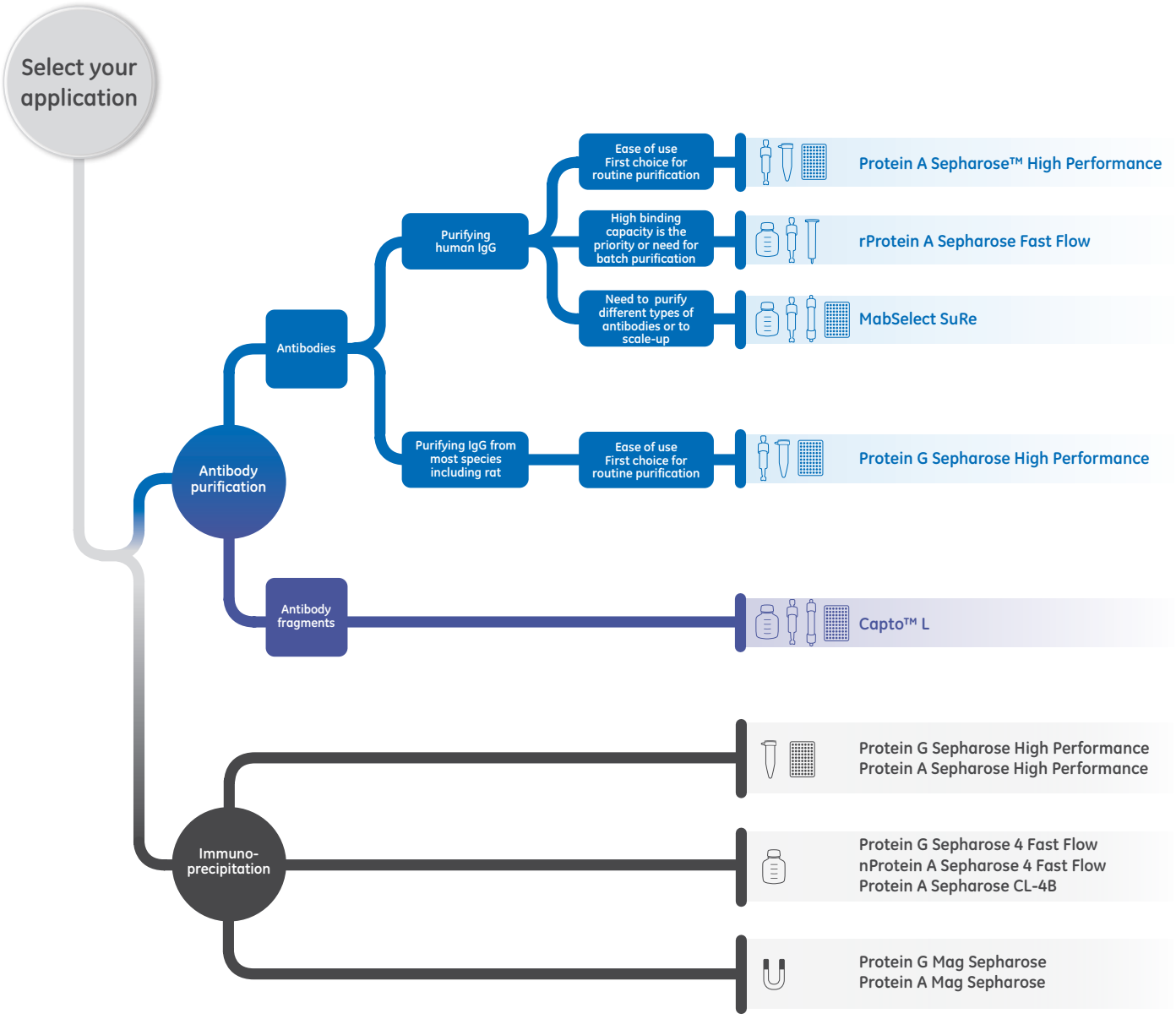
Fluorescence occurs when molecules called fluorophores absorb light. In their ground state, fluorophores do not emit light, but when subjected to light (excitation) their energy levels are raised to a brief but unstable excited state. As fluorophores return to their ground state, they release light at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



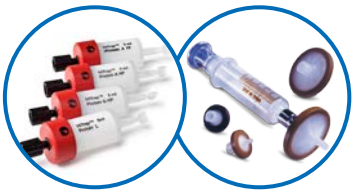
Products

Select your antibody purification 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™, and 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 purification 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



Ordering information for Whatman syringe filters

Membrane	Format	Description	Hold up volume	Pack size	Item
Polyethersulfone (PES)*	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)†	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
	30 mm, 0.2 µm	SPARTAN syringe filters, RC§	–	500	10463062
	30 mm, 0.45 µm	SPARTAN syringe filters, RC§		500	10463052

* *PES - Hydrophilic membrane. Particularly suitable for filtration of serum, plasma and tissue culture solutions.*

† *RC - Hydrophilic membrane. Very good chemical resistance to a broad range of solvents including all common solvents used in HPLC (methanol, acetonitrile, water); also exhibits low levels of non-specific protein binding.*

‡ *The GD/X range is specifically designed for high particulate loaded samples. Constructed of a pigment-free polypropylene housing with a prefiltration stack of glass microfiber media, these filters allow you to filter even the most difficult samples with less hand pressure. GD/X syringe filters can process three to seven times more sample volume than unprotected membranes.*

§ *SPARTAN syringe filters support reproducible results. Use for any application requiring a chemically resistant, hydrophilic, low protein-binding membrane.*

Ordering information for antibody purification 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
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

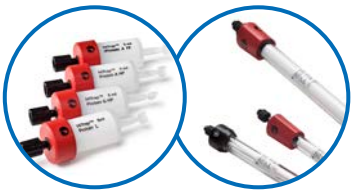
Ordering information for antibody fragment purification columns

Resin and dynamic binding capacity	Format	Description	Column volume	Pack size	Item
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

Ordering information for 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 at **gelifesciences.com/sampleprep** or in the VivaSpin data file.



Ordering information for 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

Ordering information for SEC columns

Resin and fractionation range	Format	Description	Sample volume	Column volume	Pack size	Item
Superdex 200 Increase 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 High Resolution 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 High Resolution 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

Ordering information for desalting columns

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*	0.1–1.5 mL*	5 mL	5 columns	17140801
		HiTrap Desalting, 1 × 5 mL*	0.1–1.5 mL*	5 mL	1 column	29048684
Sephadex G-25 Fine Exclusion limit M_r 5000	HiPrep column	HiPrep 26/10 Desalting	≤ 15 mL*	53 mL	1 column	17508701
Sephadex G-25 Medium Exclusion limit M_r 5000	Gravity flow column	PD-10 Desalting Column†	1.0–2.5 mL†	8.3 mL	30 columns	17085101
		PD MidiTrap G-25‡	0.5–1 mL	3.5 mL	50 columns	28918008
		PD MiniTrap G-25‡	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

* *HiTrap and HiPrep: up to 3 columns can be easily connected in series to increase the sample volume if needed.*

† *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).*

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

Ordering information for ÄKTA 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.

System	Component	Description	Flow rate	Maximum pressure	Item
ÄKTA start 	ÄKTA start system	ÄKTA start, our simplest ÄKTA system, is an affordable, easy-to-use protein purification system that allows you to automate manual protein purification procedures in academic and educational labs. Save time, minimize labor, and learn how to use automated chromatography.	0.5 to 5 mL/min	0.5 MPa	29022094
	Frac 30	ÄKTA start can be equipped with Frac30, a round fraction collector that is controlled through either the ÄKTA start touchscreen display or through UNICORN start. 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.	–	–	29023051
	UNICORN start 1.1	UNICORN start allows you to design runs, operate the ÄKTA start instrument, and to evaluate and share results. UNICORN start 1.1 is verified for the following operating systems: • Windows® 7 Professional SP1 • Windows 10 Professional	–	–	29237234
ÄKTA pure 	ÄKTA pure 25 L	The ÄKTA pure system is modular with valves, monitors, and columns mounted on the forward facing wet side of the system. The design allows easy interaction with the instrument components. Additional components such as valves, monitors, and sensors from the wide range of optional modules can easily be added to available positions. ÄKTA pure is equipped with either a fixed wavelength UV monitor (L systems) or a variable multiwavelength UV and visible spectrum monitor (M systems). ÄKTA pure standard components: System pump, Mixer, Injection valve, UV monitoring, Conductivity monitor.	0.001 to 25 mL/min (up to 50 mL/min during column packing)	0 to 20 MPa	29018224
	ÄKTA pure 25 L1 (V9-IAB, V9-Os)				29018225
	ÄKTA pure 25 M				29018226
	ÄKTA pure 25 M1 (V9-IAB, V9-Os)				29018227
	ÄKTA pure 25 M2 (V9-IA, V9-IB, V9-C, V9-O)				29018228
	Round Fraction Collector, F9-R	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 Collector fraction volume: 0.1 to 50 mL Spillage-free mode: DropSync	–	–	29011362
	Flexible Fraction Collector, F9-C	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), which means that samples can be collected in the format needed. Six cassettes can be loaded into the Fraction Collector, in any combination that fits the user's needs	–	–	29027743
	UNICORN 7 for Academia	This UNICORN product package is designed to meet the specific user needs in academia. It provides the flexibility to work either in your lab or remotely in the comfort of your office. UNICORN 7.0.2 is verified for the following operating systems: • Windows 7 Professional SP1 • Windows 10 Professional	–	–	29203853

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 ÄKTAmicro has been discontinued. To improve your protein purification output we recommend upgrading to ÄKTA start, ÄKTA pure or ÄKTA avant.

Please contact your GE Healthcare sales representative for further support or please visit **gelifesciences.com/AKTAlabsystems** or **gelifesciences.com/aktapure** for more details.

Ordering information for 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 600, 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 600RGB	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 **gelifesciences.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, GE Healthcare, 18103746 Edition AF (2016).
Affinity Chromatography, Vol. 2: Tagged Proteins, GE Healthcare, 18114275 Edition AF (2016).

Further recommended handbooks:

Strategies for Protein Purification, GE Healthcare, 28983331 Edition AA (2010).
Size Exclusion Chromatography: Principles and Methods, GE Healthcare, 18102218 Edition AL (2014).
Western Blotting: Principles and Methods, GE Healthcare, 28999897 Edition AC (2014).
Imaging: Principles and Methods, GE Healthcare, 29020301 Edition AA (2012).

Please visit **gelifesciences.com/ProteinHandbooks** for downloads and more details.

Join ÄKTA club for free today

Access to information about your ÄKTA system has never been so easy.

ÄKTA club is an online user community for ÄKTA system users enabling easy and quick access to trustable information about ÄKTA systems and protein purification.

Stay connected within the community of ÄKTA system users.

Connect to forums on ÄKTA club. Share your experience and results, engage in inspiring discussions, and learn from peers and GE experts.

gelifesciences.com/AKTAclub

Get further guidance on product selection

Selection Guides for download

Selection guide: *Columns and resins for antibody purification and immunoprecipitation,* GE Healthcare, 28935197, Edition AB (2016).
Selection guide: *Size exclusion chromatography columns and media,* GE Healthcare, 18112419, Edition AI (2016).
Selection guide: *Your guide to chromatography media,* GE Healthcare, 29167217, Edition AA (2015).
Poster: *Guide to modern BioProcess™ chromatography resins,* GE Healthcare, 29231394, Edition AA (2016).
Selection guide: *Prepacked chromatography columns for ÄKTA systems,* GE Healthcare, 28931778, Edition AE (2011).

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 **gelifesciences.com/purify**

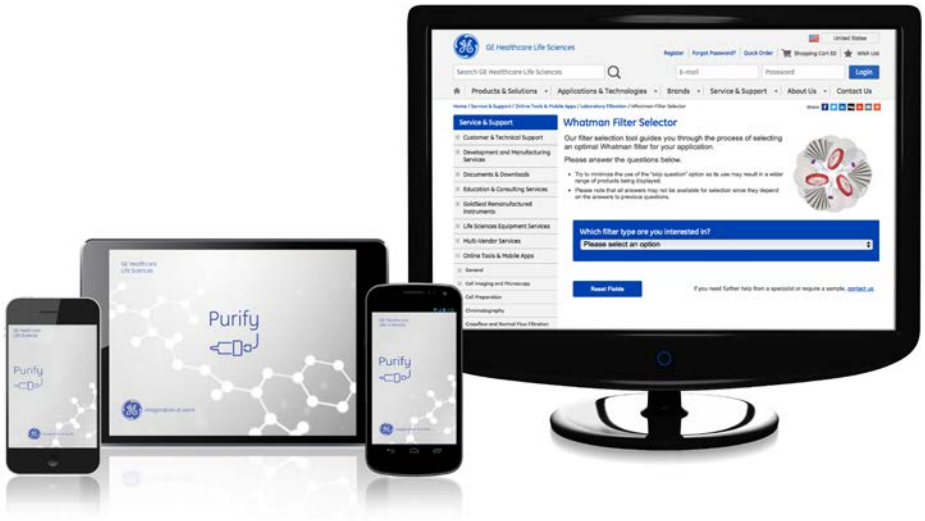
ÄKTA system accessories app
This guide will help you to quickly select the correct ÄKTA system accessories (tubing, frac racks, column holders, connectors and fittings). Pictures of different accessories will help you to identify the item you need. To support you in the ordering process there is an email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

Download the app on **gelifesciences.com/aktaapps**

Whatman filter selector
The Whatman filter selector from GE Healthcare's Life Sciences 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 **gelifesciences.com/whatmanselector**



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