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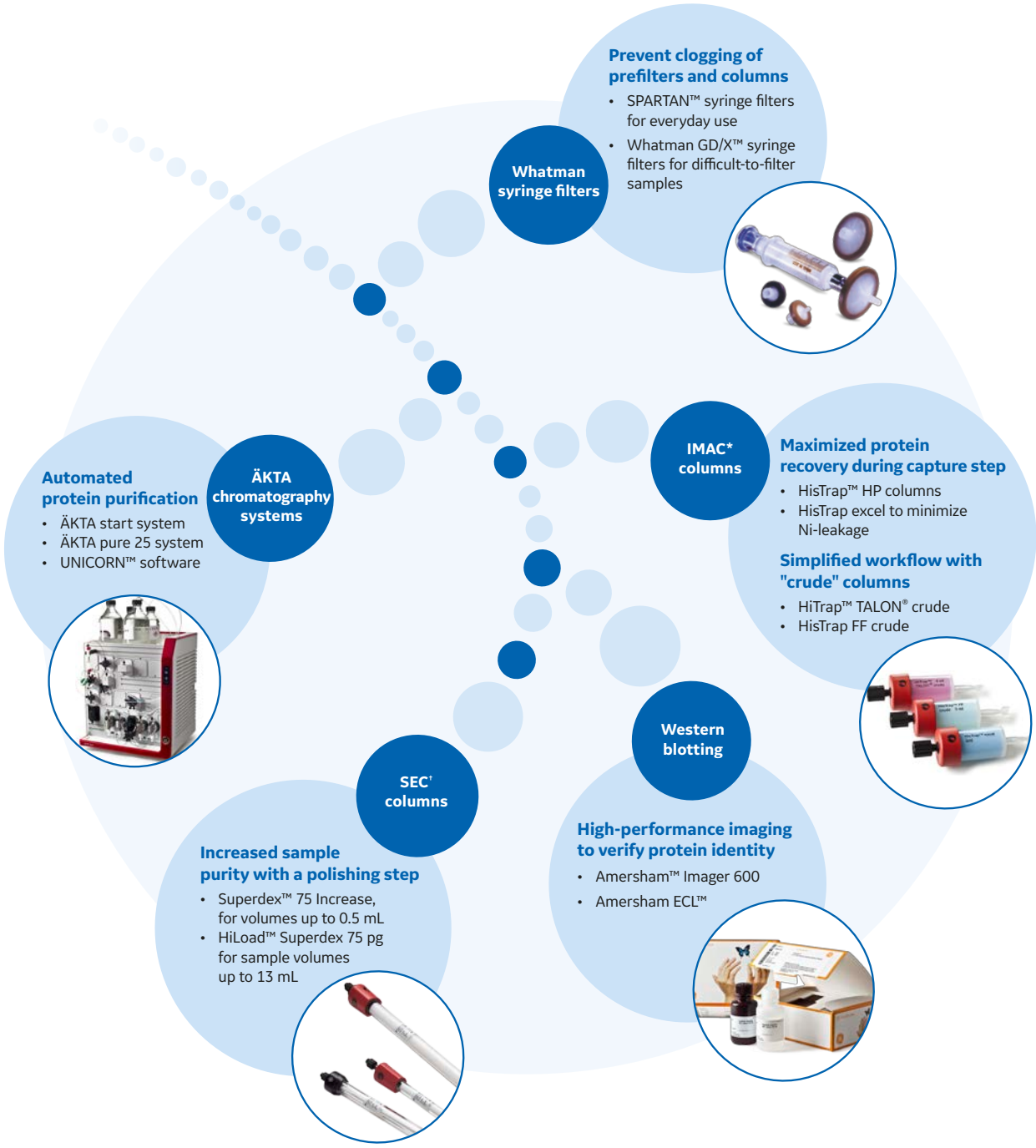
Introduction to histidine-tagged purification and analysis

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 Metal Ion Affinity Chromatography (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.

How does IMAC work?

IMAC purification begins with equilibration of the column with a binding buffer containing a low concentration of imidazole. The concentration of imidazole depends on the selected chromatography resin (can be found in the instruction for the specific resin). The imidazole binds to the immobilized metal ion and becomes the counter ligand. The sample should be adjusted to the same imidazole concentration as the binding buffer before being loaded on the column. Proteins with histidines bind to the resin/prepacked column while displacing the imidazole counter ligands. The resin/prepacked column is washed using the binding buffer. Elution of bound proteins is performed using a gradient of imidazole up to 100 to 500 mM or by step elution (Figs 2 and 3).

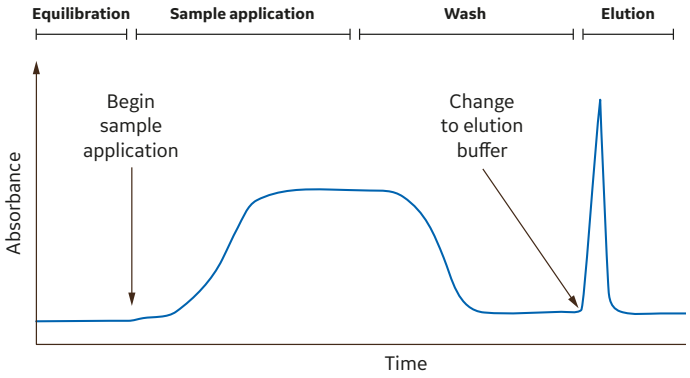


Fig. 2. General purification workflow for his-tagged proteins under native conditions. All metal-ion-charged chromatography resins follow the same workflow although the imidazole concentrations in the binding and elution buffer may differ. The workflow is also valid for purification under denaturing conditions (including 8 M urea or 6 M Gua-HCl).

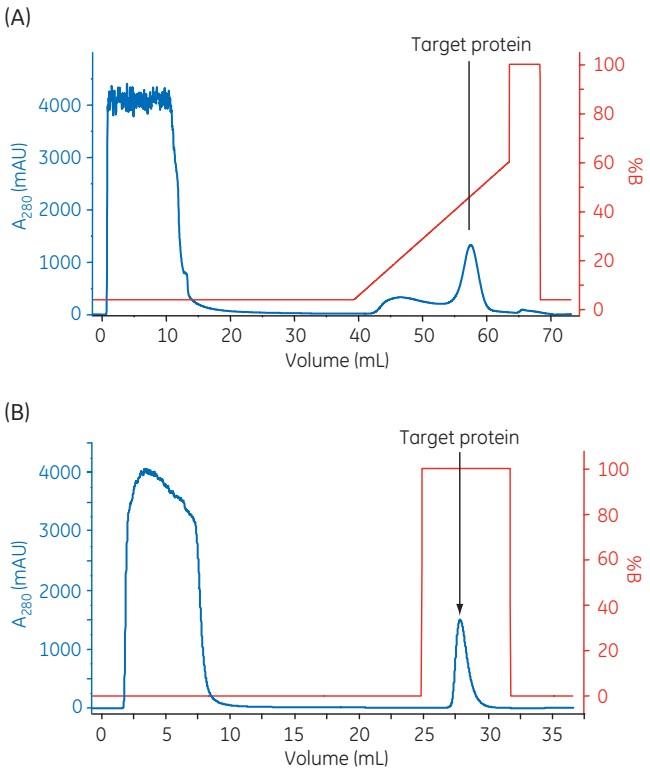


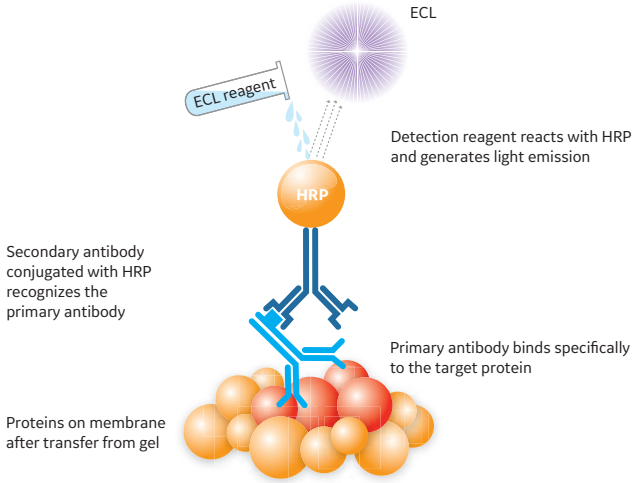
Fig. 3. Typical IMAC purification with (A) Gradient, or (B) Step elution.

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.

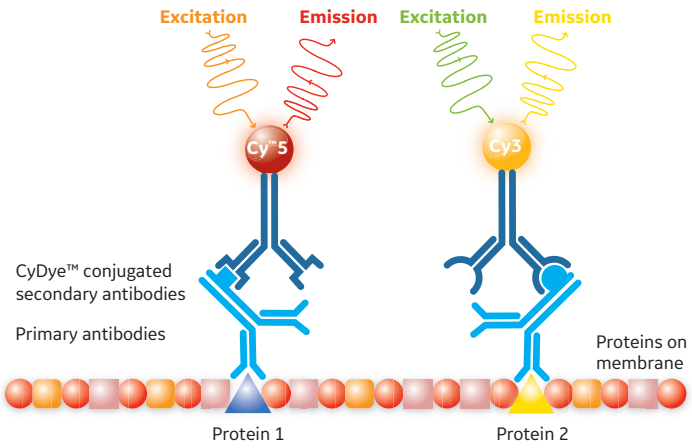


Fluorescence

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 IMAC resin

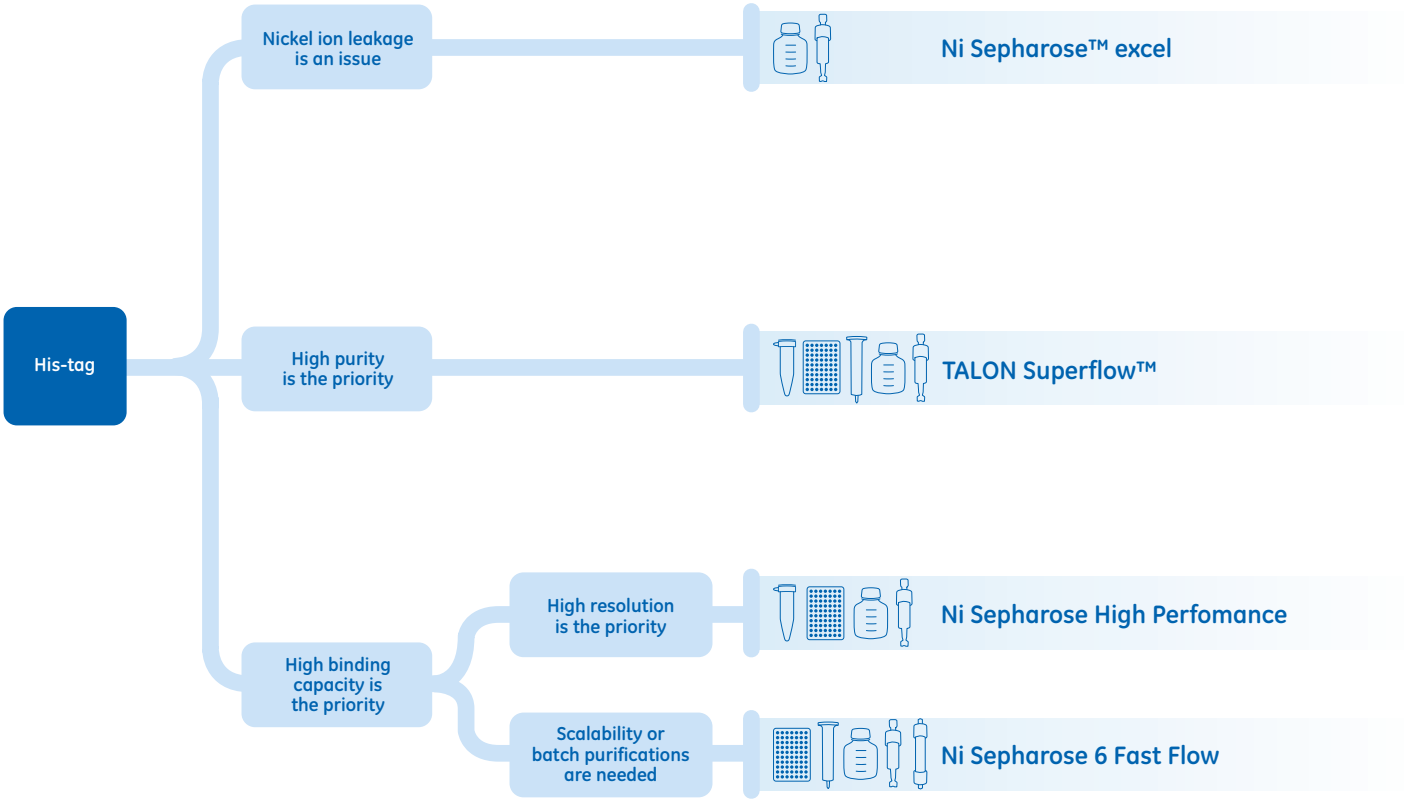


Fig. 4. Selection guide to quickly identify the most suitable resin for the purification of your his-tagged protein.

Select your 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™, and PD10	Lab pack	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	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 IMAC columns

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*	–	–	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

* *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)*

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 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.

Ordering information for SEC columns

Resin	Format	Description	Sample volume	Column volume	Pack size	Item
Superdex 75 Increase 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

Ordering information for desalting columns

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*	0.1 to 1.5 mL*	5 mL	5 columns	17140801
		HiTrap Desalting, 1 × 5 mL*	0.1 to 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 to 2.5 mL	8.3 mL	30 columns	17085101
		PD MidiTrap G-25†	0.5 to 1 mL	3.5 mL	50 columns	28918008
		PD MiniTrap G-25†	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

* *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 x columns stand, 4 x PD-10 spin adaptors, 1 x Buffer tray, 30 x 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.

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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**

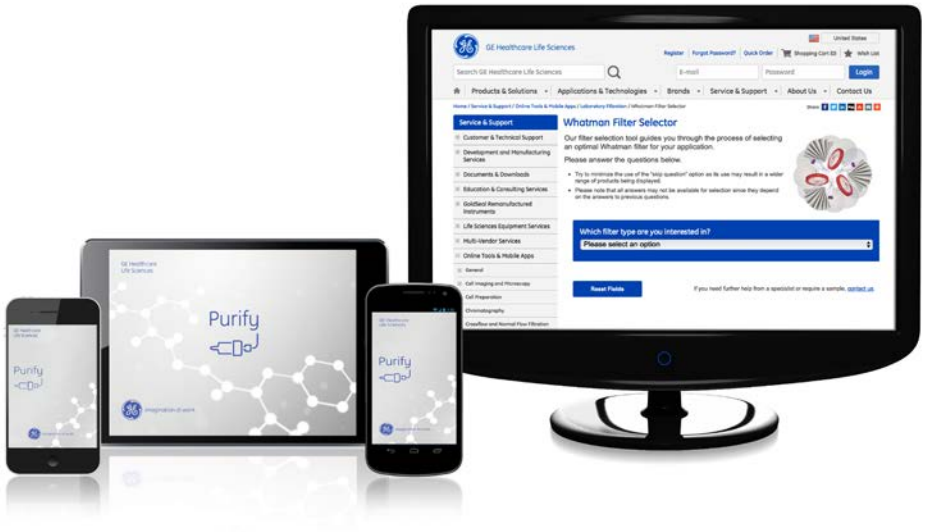
ÄKTA system accessories app
This guide will help you to quickly select the correct ÄKTA system accessories (tubing, frac racks, column holders, connectors and fittings). Pictures of different accessories will help you to identify the item you need. To support you in the ordering process there is an email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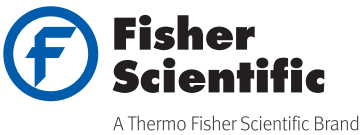

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