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Lab recharge 2020

Life science research solutions for biotech

Isolation and sequencing | Cell culture | Sample prep | Purification | Analysis

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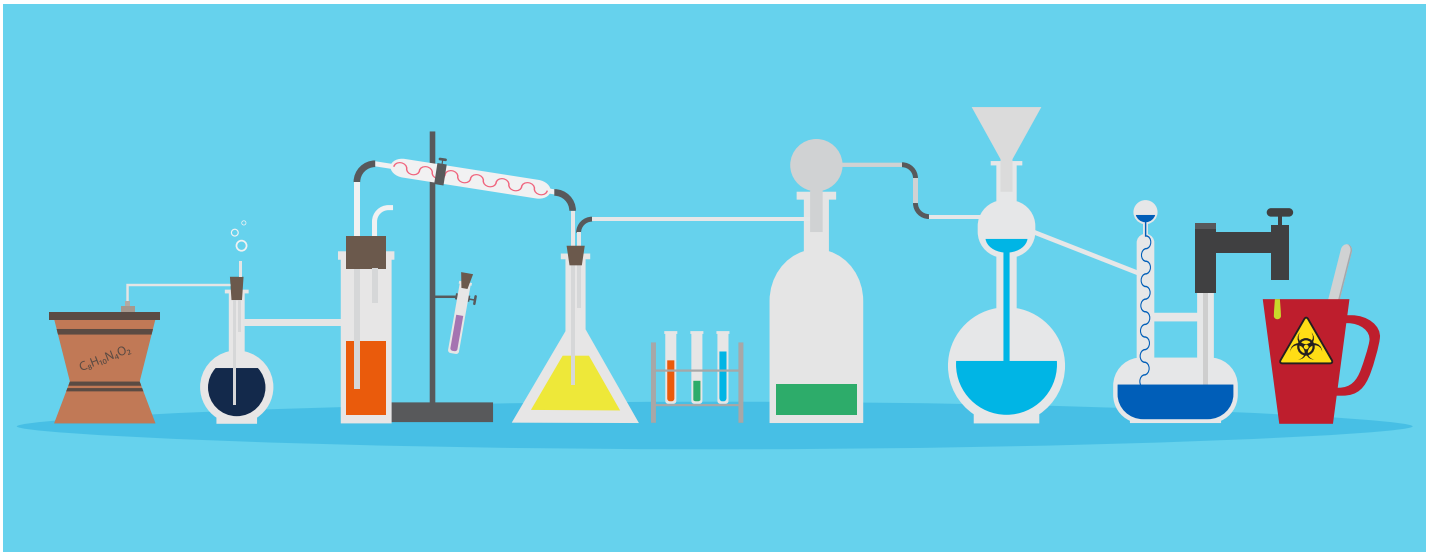

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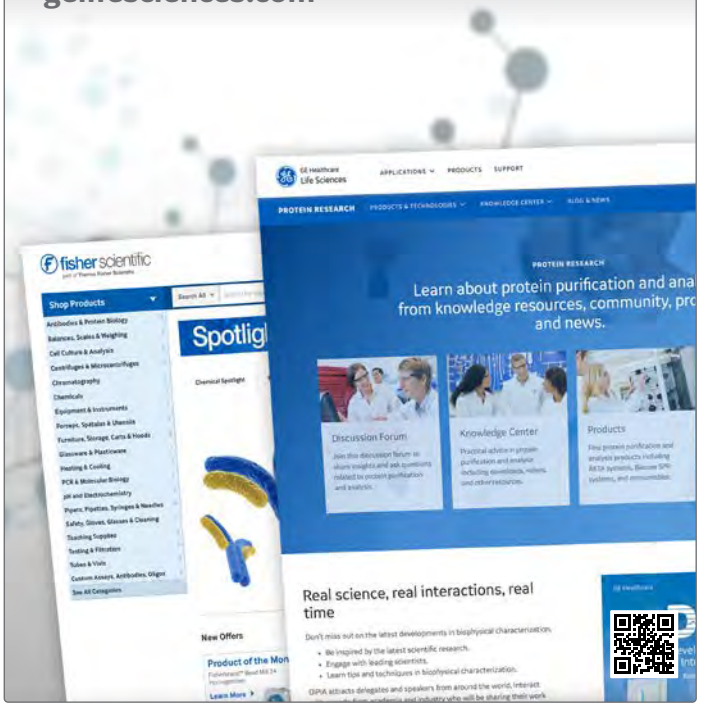

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Magbeads 101: A guide to choosing and using magnetic beads

Magnetic beads (or superparamagnetic particles) are versatile little tools for easy and effective isolation of biomolecules. Use this guide to compare the different surface chemistries and find the type for your application.

What are magnetic beads?

Magnetic beads are made up of tiny (20 to 30 nm) particles of iron oxides, such as magnetite (Fe_3O_4), which give them superparamagnetic properties.

Superparamagnetic beads are different to more common ferromagnets in that they exhibit magnetic behavior only in the presence of an external magnetic field. This property is dependent on the small size of the particles in the beads, and enables the beads to be separated in suspension, along with anything they are bound to. Since they don't attract each other outside of a magnetic field, they can be used without any concern about unwanted clumping.

There are many types of magnetic beads available. Different surface coatings and chemistries give each type of bead its own binding properties, which can be used for magnetic separation (isolation and purification) of nucleic acids, proteins, or other biomolecules in an easy, effective, and scalable way.

This ease-of-use makes them automation friendly and well suited for a range of applications, including sample preparation for next generation sequencing (NGS) and PCR, protein purification, molecular and immunodiagnostics, and even magnetic activated cell sorting (MACS), among many others. They also ease some of the challenges associated with extracting nucleic acids from different sample types.

What is magnetic separation?

Magnetic separation uses a magnetic field to separate micrometer-sized paramagnetic particles from a suspension. In molecular biology, magnetic beads provide a simple and reliable method of purifying various types of biomolecule, including genomic DNA, plasmids, mitochondrial DNA, RNA, and proteins.

For example, under optimized conditions, DNA selectively binds to an appropriately-coated bead surface, leaving contaminants in solution. You can then use this purified DNA directly in molecular biology applications.

A key advantage to using magnetic beads is that you can isolate nucleic acids and other biomolecules directly from a crude sample, and from a variety of different types of sample, with minimal processing. This sets magnetic beads apart from other methods of nucleic acid isolation, which might have different protocols for different types of sample, and involve more hands-on time.

How does magnetic bead DNA extraction work?

Magnetic beads have been around in one form or another for decades. Their potential in nucleic acid purification was recognized in the 1990's, as demonstrated by the US patent: "DNA purification and isolation using magnetic particles". The approach, largely unchanged since, relies on using magnetic beads with a coating that can bind nucleic acids reversibly by just adjusting buffer conditions (Fig 1).

After binding DNA, an external magnetic field attracts the beads to the outer edge of the containing tube, immobilizing them. While the beads are immobilized, the bead-bound DNA is retained during the washing steps. Adding elution buffer, and removing the magnetic field then releases the DNA as a purified sample, ready for quantitation and analysis.

This approach removes the need for vacuum or centrifugation, which minimizes stress or shearing forces on the target molecules, requires fewer steps and reagents than other DNA extraction protocols, and is amenable to automation in 24, 96, and 384-well plates.

Magnetic particles are added to sample and bind to target molecule

Magnetic particles are captured and remainder of sample is washed away

Target molecule is released from magnetic particles for further analysis

Fig 1. Overview of magnetic bead-based DNA extraction using Sera-Mag™ beads.

*Read full article and blogs **here**.*

Featured products

Sera-Mag SpeedBeads and Sera-Mag Streptavidin-Coated Magnetic Particles

Provide a high biotin-binding capacity along with a strong affinity for targeted, biotin labeled molecules. Available with low (2500 to 3500 pmol/mg), medium (3500 to 4500 pmol/mg) or high (4500 to 5500 pmol/mg) nominal biotin binding capacities for optimizing assay development.

Sera-Mag SpeedBeads and Sera-Mag Carboxylate-Modified Magnetic Particles

Combine a fast magnetic response time and high binding capacity with a large surface area, high sensitivity, stability, physical integrity, and fast reaction kinetics. Typical applications include sample preparation, proteomics, nucleic acid isolation, and immunoassay applications. Carboxylic groups on the surface permit easy covalent coupling to biomolecules of interest using convenient carbodiimide chemistry.

SeraSil-Mag silica coated superparamagnetic beads

NEW PRODUCT

For nucleic acid isolation deliver high purity DNA extraction for highly sensitive applications where sample is scarce. These beads provide an optimal surface for nucleic acid binding with high performance and low background. High magnetization (60 emu/g) and strong binding capacity giving fast magnetic response (~5 secs) and shorten time required for magnetic steps during isolation.

Click ***here*** for further details on the above products and related specifications.

Ordering information

Chemistry	Format	Description	Volume	Pack size	Item
Nucleon resin	Kit	Nucleon BACC1	25 preps	1/pk	RPN8501
Nucleon resin	Kit	Nucleon HT	50 preps	1/pk	RPN8509
Nucleon resin	Kit	Nucleon BACC3	50 preps	1/pk	RPN8512
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBeads Streptavidin-Blocked	100 mL	1/pk	21152104010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBeads Streptavidin-Blocked	1 mL	1/pk	21152104011150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Carboxylate-Modified Magnetic Particles (Hydrophylic)	100 mL	1/pk	24152105050350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 2500 to 3500 (low) pmol per mg	5 mL	1/pk	30152103010150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 2500 to 3500 (low) pmol per mg	100 mL	1/pk	30152103010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 3500 to 4500 (med) pmol per mg	5 mL	1/pk	30152104010150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 3500 to 4500 (med) pmol per mg	100 mL	1/pk	30152104010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 4500 to 5500 (high) pmol per mg	5 mL	1/pk	30152105010150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 4500 to 5500 (high) pmol per mg	100 mL	1/pk	30152105010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Carboxylate-Modified Magnetic Particles (Hydrophobic)	15 mL	1/pk	44152105050250
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBead Carboxylate (Hydrophylic)	15 mL	1/pk	45152105050250
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBead Carboxylate (Hydrophobic)	15 mL	1/pk	65152105050250
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBead Carboxylate (Hydrophobic)	100 mL	1/pk	65152105050350
Silica coated beads	Kit	SeraSil-Mag 400 ***NEW***	5 mL	1/pk	29357369
Silica coated beads	Kit	SeraSil-Mag 400 ***NEW***	60 mL	1/pk	29357371
Silica coated beads	Kit	SeraSil-Mag 700 ***NEW***	5 mL	1/pk	29357373
Silica coated beads	Kit	SeraSil-Mag 700 ***NEW***	60 mL	1/pk	29357374

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Ficoll-Paque™ PREMIUM density gradient media

Ficoll-Paque PREMIUM products are a range of sterile, ready-to-use density gradient media for the preparation of mononuclear cells. All Ficoll-Paque PREMIUM products have low endotoxin levels (< 0.12 EU/mL) and are manufactured under a Quality Management System certified to ISO 13485 and to the guidelines outlined in EU GMP Annex 1: Manufacture of Sterile Medicinal Products (1). Ficoll-Paque PREMIUM products are available in densities of 1.073, 1.077, and 1.084 g/mL for the preparation of different density preparations of mononuclear cells from peripheral blood, bone marrow, umbilical cord blood, and placental tissue. Mononuclear cell isolation can be automated and functionally closed by using Sepax™ technology (2, 3).

Features

- Manufactured within a quality management system certified to ISO 13485.
- Meet USP <1043> 'ancillary materials for cell, gene, and tissue engineered products', within the responsibilities applicable to a supplier (4).
- Suitable for in vitro applications.
- Sterile, ready-to-use reagent.
- Low levels of endotoxin (< 0.12 EU/mL) secured and tested.

Classical Ficoll-Paque PREMIUM with a density of 1.077 g/mL was developed from Ficoll-Paque PLUS, which is based on Ficoll™ PM400 (polysucrose) and sodium diatrizoate and has a more than 40 yr track record for large- or small-scale purification of mononuclear cells from human peripheral blood. All Ficoll-Paque PREMIUM products differ from Ficoll-Paque PLUS in that they are manufactured under a Quality Management System certified to ISO 13485 and to the guidelines outlined in EU GMP Annex 1: Manufacture of Sterile Medicinal Products (3). These require stringency in validation and documentation of manufacturing procedures.

Applications

Ficoll-Paque PREMIUM

Ficoll-Paque PREMIUM has a density of 1.077 g/mL and is optimized for the isolation of mononuclear cells from human peripheral blood by using a simple and rapid centrifugation technique developed by Bøyum et al. (5). The medium can also be used for the isolation of human mononuclear cells from other sources, including bone marrow and umbilical cord blood.

Separation of normal human peripheral blood by the recommended protocol typically yields a mononuclear cell preparation with:

- 95% ± 5% mononuclear cells present in the separated fraction
- > 90% viability of the separated cells
- 60% ± 20% recovery of the mononuclear cells present in the original blood sample
- 3% ± 2% granulocytes
- 5% ± 2% red blood cells

Save time in the lab by using our Percoll™ Calculator

Click ***here*** to use it.

*Read full article **here.***

Featured products

Ficoll-Paque PLUS and Ficoll-Paque PREMIUM

Table comparing the different Ficoll products.

Parameter	Ficoll-Paque PLUS	Ficoll-Paque PREMIUM	Ficoll-Paque PREMIUM 1.073	Ficoll-Paque PREMIUM 1.084
Application	Isolation of human mononuclear cells for *in vitro* studies. For research use only	Isolation of mononuclear cells from human peripheral blood, bone marrow, and umbilical cord blood	Isolation of lower-density human mononuclear cells (e.g. mesenchymal stromal cells or monocytes)	Isolation of a broad range of human mononuclear cells including those of a higher density and for separating blood cells from mice or rats
Density	1.077 g/mL	1.077 g/mL	1.073 g/mL	1.084 g/mL
Osmolality	–	288 to 310 mOsm/kg	276 to 298 mOsm/kg	322 to 344 mOsm/kg
Regulatory	–	Manufactured under a Quality Management System certified to ISO 13485		
Physical state	Liquid			
Endotoxin activity max.	< 0.12 EU/mL			
pH range	5.5 to 7.5			
Color	Colorless to slight yellow			
Sterility	Autoclave steam sterilization with sterility assurance level (SAL) of 10^{-6}			
Estimated shelf life/ Stability	At least 3 yr from manufacture date under recommended storage conditions. Deterioration of Ficoll-Paque products is indicated by the appearance of a yellow color or particulate material in the solution			
Storage conditions	4°C to 30°C and protected from light			

Percoll and Percoll PLUS

Are silica-based colloidal media for cell separation by density gradient centrifugation

Percoll offers:

- Low osmolality: can easily be adjusted with physiological saline, cell culture medium, or sucrose to give gradients that are iso-osmotic throughout
- Low viscosity resulting in rapid formation of gradients and particle separation at low centrifugal forces
- Support through extensive research use: Thousands of publications on Percoll in scientific journals
- Formation of either continuous preformed or self-generated gradients by centrifugation at moderate speeds

Percoll PLUS offers:

- Low endotoxin levels (max. 2 EU/mL)
- Absence of toxicity for cells and very low chemical reactivity
- Low osmolality: can easily be adjusted with physiological saline, other balanced salt solutions, or cell culture media, to give gradients that are iso-osmotic throughout
- Low viscosity resulting in rapid formation of gradients and particle separation at low centrifugal forces

Ordering information

Product type	Format	Description	Pack size	Item
Sucrose Polymer	Bag	Ficoll PM400	5 kg	17030005
Sucrose Polymer	Bag	Ficoll PM400	500g	17030050
Media	Bottle	Percoll	1 L	17089101
Media	Bottle	Percoll	250 mL	17089102
Media	Bottle	Ficoll-Paque PLUS	6 × 100 mL	17144002
Media	Bottle	Ficoll-Paque PLUS	6 × 500 mL	17144003
Media	Bottle	Percoll PLUS	1 L	17544501
Media	Bottle	Percoll PLUS	250 mL	17544502
Media	Bottle	Ficoll-paque PREMIUM 1.084	6 × 100 mL	17544602
Media	Bottle	Ficoll-paque PREMIUM 1.073	6 × 100 mL	17544652

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Serum alternatives to fetal bovine serum in cell culture

Serum is often a necessary component of cell culture. Fetal bovine serum (FBS) has long been the first serum of choice for researchers. Although FBS performs well, there are circumstances where FBS replacements might offer advantages with regard to cost of sera, variability in supply, lot-to-lot variability in composition, or performance with specific cell types. This study examines the performance of FBS and seven serum alternatives with six cell lines.

Key concepts and findings

- Comparisons were made with the FBS control condition as a base standard, using a ratio of cell counts.
- Available FBS replacements are shown to work with six cell lines.
- The FBS replacements have advantages over FBS and provided equivalent or better cell growth compared with FBS (results are cell-line dependent).

Methodology

Six cell lines and eight serum types were used. All cultures were grown in T-25 cell culture flasks in 10 mL of corresponding media supplemented with 10% serum. Control FBS was prepared by pooling many lots of FBS. HyClone™ FetalClone™ sera are blends of FBS and specially processed calf serum formulated to reproduce the composition of FBS. FetalClone I, II, and III are optimized for hybridoma, CHO, and fibroblast cells, respectively. Iron-Supplemented Calf Serum is produced from formula-fed veal animal serum supplemented with physiological levels of iron, and contains high levels of transferrin. Both US and New Zealand HyClone Cosmic Calf™ sera, US and New Zealand origin, are based on Iron-Supplemented Calf Serum with additional growth promoting factors. HyClone Bovine Growth Serum is also based on Iron-Supplemented Calf serum with additional trace elements, vitamins, and growth factors.

All conditions consisted of three serum lots to test lot-to-lot consistency, except control FBS of which one lot was used, and supplementation of MRC-5 and AIF cells using Cosmic Calf, New Zealand Origin where two lots were used. Flasks were seeded at 3000–5000 cells/cm^2, incubated in 5% CO_2/95% air at 37°C, and checked daily for confluency. When any culture reached confluency, all cultures were trypsinized and counted. Cell counts were normalized to the FBS control as percentages such that the FBS control is always 1.0 or 100%. Conditions that produced more cells than the control have values greater than 1.0.

It was necessary to define a ratio at which condition performance was comparable with or better than control FBS. A value of 0.90 or 90% was chosen to accommodate experimental variations in harvesting and counting.

Results and discussion

Results were cell line-dependent with certain FBS replacements proving to be more or, sometimes, less suitable for specific cell lines. In nearly all cases, cell growth in at least one of the FBS replacements was equal to or greater than cell growth in FBS. This finding indicates that researchers have viable FBS alternatives for replacements in their cell cultures.

MRC-5 cells grew more rapidly, and thus to higher yields, in FetalClone III and Bovine Growth Serum than in FBS. In comparison with cell yields in FBS, Vero cell yields were at least as high in FetalClone II, FetalClone III, New Zealand Cosmic Calf, Iron-Supplemented Calf, US Cosmic Calf, and Bovine Growth Serum. The rate of BHK-21 cell growth was about the same in FBS, FetalClone II, FetalClone III, and U.S. Cosmic Calf Serum, while the cell growth was more rapid in Bovine Growth Serum.

FetalClone II is optimized for CHO cells, as are the Cosmic Calf sera. Supplementation with FetalClone II, FetalClone III, New Zealand Cosmic Calf and US Cosmic Calf each resulted in higher yields of CHO cells than did FBS. Bovine Growth Serum performed comparably to FBS.

AIF cells were used as a model for conventional hybridoma cell lines. All sera tested supported hybridoma cell growth rates equal to or higher than with FBS. However, for many hybridoma applications, the lower IgG levels in FBS and FetalClone I make these the preferred sera for monoclonal antibody production.

NS0 cultures in FetalClones I, II, III, New Zealand Cosmic Calf, Iron-Supplemented Calf, and US Cosmic Calf serum showed growth equal to or better than that of growth in FBS.

Study conclusions

This study has shown that multiple sera are available as potential replacements for FBS in cell culture. A variety of mammalian cell types (fibroblasts, hybridoma, myeloma) were used in the study, and each type was shown to have a potential FBS replacement in at least one bovine calf-based serum. Some advantages of the tested calf-based sera compared with FBS are lower cost, higher availability, and perhaps more consistent component levels due to the methods used in the veal industry. Animal age at time of slaughter, stress on the animals, breed, and diet are factors that can contribute to the consistent component levels in calf sera compared with the same composition in fetal bovine serum.

Request a copy of data file 29194816 AA—Serum alternatives to fetal bovine serum in cell culture

Featured products

HyClone FetalClone I, II, III

FetalClone engineered serum products are economical alternatives to fetal bovine serum (FBS), commonly used in bioprocessing applications as a supplement to enrich cell culture performance. FetalClone products have demonstrated performance with a variety of cell lines, including hybridomas, CHO, BHK-21, NS0, MRC-5, and Vero cells.

HyClone Cell Culture Media for monoclonal antibody (mAb) and recombinant protein production

These serum-free basal media are designed to be utilized with common protein-producing cell lines, such as CHO and HEK293. See below table for animal-derived component-free (ADCF), chemically-defined (CD), and protein-free formulations for use with your cell line of interest.

CHO cells	PF-CHO	SFM4CHO	CDM4CHO	
	HyCell™ CHO	HyCell TransFx-C	ActiPro™	ActiSM™
Hybridoma/ myeloma	CDM4MAb	CDM4PERMAb	SFM4MAb	CDM4NS0
Insect cells	SFX-Insect	SFM4Insect		
Viral vaccines	SFM4Megavir	CDM4Avian		
HEK293/PER.C6	CDM4PerMAb	CDM4HEK293	SFM4HEK293	TransFx-H
Stem cells	HyCell STEM	AdvanceSTEM™		

HyClone Classical Media	
BME/EBSS	MEM/EBSS
DMEM	DMEM/F12
Ham's F10	Ham's F12
Iscove's (IMDM)	Leibovitz (L-15)
McCoy's 5A	Medium 199
MEM	RPMI 1640

Try Whatman™ syringe filters to prepare your sample.

Click **here** for further information.

Ordering information

Product type	Format	Description	Volume	Pack size	Item
Serum	Bottle	FetalClone II	500 mL	1/pk	SH30066.03
Serum	Bottle	FetalClone I	500 mL	1/pk	SH30080.03
Serum	Bottle	FetalClone III	500 mL	1/pk	SH30109.03
Media	Bottle	ADCF MAb	500 mL	1/pk	SH30349.01
Media	Bottle	SFM4HEK293, with L-Glutamine	1000 mL	1/pk	SH30521.02
Media	Bottle	SFM4CHO without L-Glutamine, with 2.2 g/L Sodium Bicarbonate	1000 mL	1/pk	SH30548.02
Media	Bottle	SFM4Transfx-293 without L-Glutamine	1000 mL	1/pk	SH30860.02
Specialty Media	Bottle	HyCell TransFx-H, HEK293 Transient transfection medium	1000 mL	1/pk	SH30939.02
Specialty Media	Bottle	HyCell TransFx-C, CHO Transient transfection medium	1000 mL	1/pk	SH30941.02
Supplement	Bottle	Cell Boost 1 liquid	500 mL	1/pk	SH31113.01
Supplement	Bottle	Cell Boost 2 liquid	500 mL	1/pk	SH31114.01
Supplement	Bottle	Cell Boost 3 liquid	500 mL	1/pk	SH31115.01
Supplement	Bottle	Cell Boost 4 liquid	500 mL	1/pk	SH31116.01
Supplement	Bottle	Cell Boost 5 liquid	500 mL	1/pk	SH31117.01
Supplement	Bottle	Cell Boost 6 liquid	500 mL	1/pk	SH31118.01
Supplement	Bottle	Cell Boost 7a liquid	500 mL	1/pk	SH31119.01
Supplement	Bottle	Cell Boost 7b liquid 100 ml	100 mL	1/pk	SH31120.01
Supplement	Bottle	Cell Boost 7b liquid 500 ml	500 mL	1/pk	SH31120.02

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Save time in HPLC prep

Sample filtration protects your HPLC instrument and column while preserving data quality. Read our tips on using multilayer and all-in-one filter units to save time and improve lab efficiency.

If you analyze large numbers of samples using high-performance liquid chromatography (HPLC), sample preparation can take up a lot of your time. Filtering samples before HPLC can help avoid frit clogging while maintaining data quality.

So, what can you do to simplify and speed up the process? Read on to find out!

Try a stacked syringe filter

Syringe filtration often involves aspirating the sample, fitting a particle filter, filtering into an autosampler vial, capping, and finally transferring the vial to an auto-sampler. You might repeat this process dozens of time a day, depending on your circumstances.

If you have difficult-to-filter samples, you might find that high particulate samples can take more time to filter. To help with this, stacked filter devices have multiple layers of filtration, starting with larger pore sizes and going down to the desired pore size.

This approach traps large particles first, and successively traps smaller particles. The device does not get clogged as easily as devices with a single membrane, making filtration faster and easier.

Go syringeless

If your samples are reasonably easy to filter, a syringeless filter option simplifies the process greatly.

Using a standard syringe filter involves at least four individual components, five if you include the initial sample storage vial. When you have dozens (or hundreds!) of samples to filter, the multi-step workflow is time consuming and can lead to sample loss.

In a syringeless filter, the filter membrane, pre-filtration chamber, post-filtration storage vial, and cap are all part of one device. This design streamlines HPLC sample prep and minimizes the number of consumables. Filtration can be performed 3 times faster than with syringe filters.

Using a syringeless filter means that you only need to add the sample to the outer chamber, place the plunger, and push. The inner storage vial holds your filtered sample ready for analysis, so it can go directly into your autosampler. Construction can be either polypropylene or glass and the vial can be either clear or amber colored depending on the requirements around your sample.

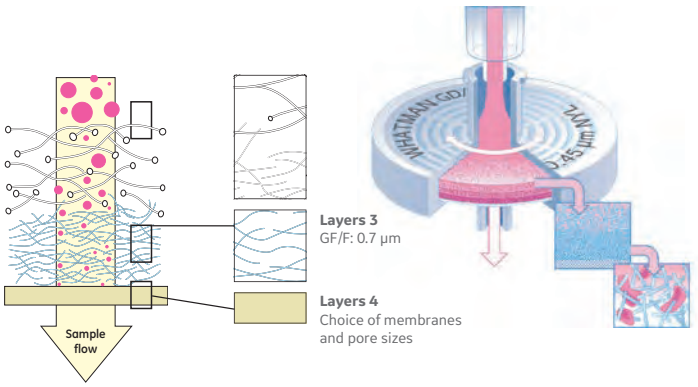
Broaden your solvent compatibility

When your lab prepares a wide variety of sample types using different solvents for HPLC analysis, identifying appropriate membrane materials can be time-consuming. Different materials might be more or less suitable for a given sample based on chemical compatibility and solvent resistance.

If you want to make filtration easier, you could try out a material with broad solvent compatibility. Regenerated cellulose (RC), for example, is well suited for both hydrophilic and hydrophobic solvents. Using RC for most or even all your samples can reduce time spent researching and selecting materials.

Use tools to boost throughput

A multi-compressor can save time when using syringeless units. Filtering multiple samples simultaneously with a dedicated tool can also reduce hand strain.



Whatman GD/X™ stacked syringe filter

Mini-UniPrep™ syringeless filters

Try our Whatman **Filter Selector Tool** to find out if you are using the most appropriate filtration solution for your samples. Click ***here*** to get there.

*For more information please visit **eu.fishersci.com***

Featured products

Mini-UniPrep syringeless HPLC filters

Whatman Mini-UniPrep syringeless filters integrate an autosampler vial, filtration membrane, plunger, and cap/septa into one consumable product. They are built for fast and easy HPLC/UHPLC sample preparation.

- 0.2 µm and 0.45 µm pore sizes available to meet sample requirements.
- Housing options: amber to prevent photodegradation of light-sensitive samples, or translucent for easy visual inspection.
- A borosilicate glass vial version Mini-UniPrep G2 is available for eliminating plastic-based leachables that can originate from a polypropylene vial.
- Compatible with most major autosamplers for high throughput analysis.
- All-in-one filtration device for quick and cost-effective sample processing.

Whatman GD/X syringe filters

These filters are specifically designed for filtration of viscous or otherwise hard-to-filter samples with high solids content.

- High loading capacity for samples with high solids content.
- Three layer glass fibre prefiltration stack for filtering larger sample volumes with less back pressure build-up.
- Process three to seven times more sample volume than filters without prefilter.

Grade 3MM Chr cellulose chromatography papers

Grade 3MM Chr cellulose chromatography filter is a 0.34 mm thickness paper for general chromatography and electrophoresis.

- Pure cellulose produced entirely from the highest quality cotton linters with no additives of any kind.
- Manufactured and tested specifically for chromatographic techniques. This ensures the wicking capability and uniformity of capillary action that are important in chemical separations.
- Also widely used in protein and nucleic acid blotting.

Learn more about how you can add more security to your ÄKTA™ chromatography system runs by using our: Protein Prep syringe filter for ÄKTA systems – download a brochure ***here***.

Ordering information

Membrane	Format	Description	Format/pore size	Pack size	Item
RC	Non sterile	Protein Prep syringe filter for ÄKTA systems	30 mm 0.2 µm	150/pk	10463043
PTFE	Non sterile	Mini UniPrep syringeless filter	0.45 µm	100/pk	UN203NPUORG
RC	Non sterile	Mini UniPrep syringeless filter	0.2 µm	100/pk	UN203NPERC
PES	Non sterile	Mini UniPrep amber syringeless filter	0.2 µm	100/pk	UN203APEPES
RC	Non sterile	Whatman GD/X syringe filter	25 mm 0.2 µm	150/pk	6887-2502
PVDF	Non sterile	Whatman GD/X syringe filter	25 mm 0.45 µm	150/pk	6872-2504
PVDF	-	Mini-UniPrep G2 amber syringeless filter	0.2 µm	100/pk	GN203APEAQU
Cellulose	Circles	Grade 3MM Chr cellulose chromatography paper	24 mm dia	100/pk	1030-024
Absorbent	Sheets	Benchkote™ surface protector	460 mm x 570 mm	50/pk	2300-916
Absorbent	Sheets	Benchkote surface protector for ÄKTA start	310 mm x 210 mm	25/pk	2300-10064
H-PTFE	Non sterile	Puradisc syringe filter ***NEW***	25mm 0.45 µm	200/pk	6773-25043w

For your customized quotation and to place orders visit ***eu.fishersci.com***

ÄKTA start

REQUEST INFORMATION

An easy-to-learn and easy-to-use system to remove the hassles of manual protein purification

Purify tagged proteins and antibodies easily. Gain insight from real-time monitoring. Evaluate and share your results.

User friendly—Easy-to-use touchscreen display allows you to start the run at the touch of a button

Convenient—Easy transition from manual to automatic purification

Gain deeper insights—Gain valuable insights from real-time monitoring and control software

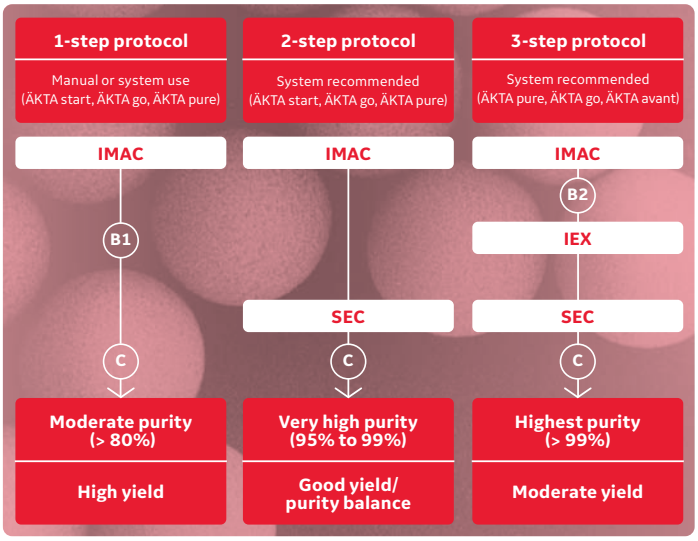
Simplify your workflow—Purify tagged proteins and antibodies easily using prepacked column

*Request information **here**.*

Protein purification protocols

Histagged protein purification protocol

Purifying histidine (his)-tagged proteins may sound easy. However, there are tips to ensure that you get the most from your his-tagged protein purification protocol, by choosing the right combination of chromatography techniques in a multistep approach. Below are examples for best practice.



IEX = ion exchange chromatography; IMAC = immobilized metal ion affinity chromatography; SEC = size exclusion chromatography; B1 = buffer exchange to remove imidazole or salts; B2 = buffer exchange to prepare for IEX; C = concentration for sample volume reduction, which may also be performed before SEC. Steps in circles are optional and are applied if necessary.

Which chromatography columns are recommended for each protein purification step?

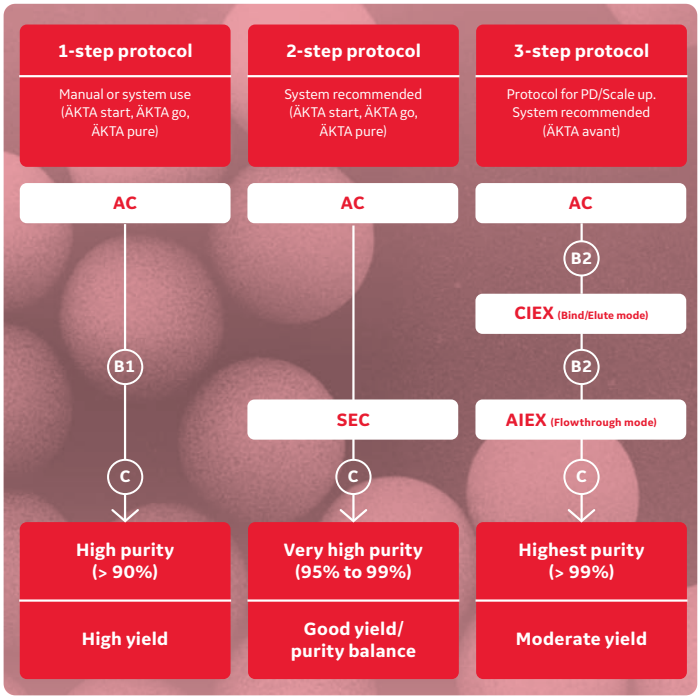
	1-step protocol	2-step protocol	3-step protocol
IMAC	HisTrap™ HP HisTrap FF crude HisTrap excel HiTrap TALON™ crude	HisTrap HP HisTrap FF crude HisTrap excel HiTrap TALON crude	HisTrap HP HisTrap FF crude HisTrap excel HiTrap TALON crude
IEX			HiTrap™ Q HP HiTrap SP HP HiTrap Capto™ Q ImpRes HiTrap Capto SP ImpRes
SEC		Superdex™ 75 Increase HiLoad™ Superdex 75 pg HiPrep™ Sephacryl™ S-100 HR HiPrep Sephacryl S-200 HR	Superdex 75 Increase HiLoad Superdex 75 pg HiPrep Sephacryl S-100 HR HiPrep Sephacryl S-200 HR

Learn more about protein purification protocols in our **Strategies for Protein Purification handbook**. Download handbook ***here.***

*For more information please visit **eu.fishersci.com***

Antibody purification protocols

Antibody purification requires the right balance between purity and yield. Typically they are challenged by two factors: (A) Capturing as many antibodies as possible and without degrading the sample and (B) removing the remaining impurities and minimizing aggregate content.



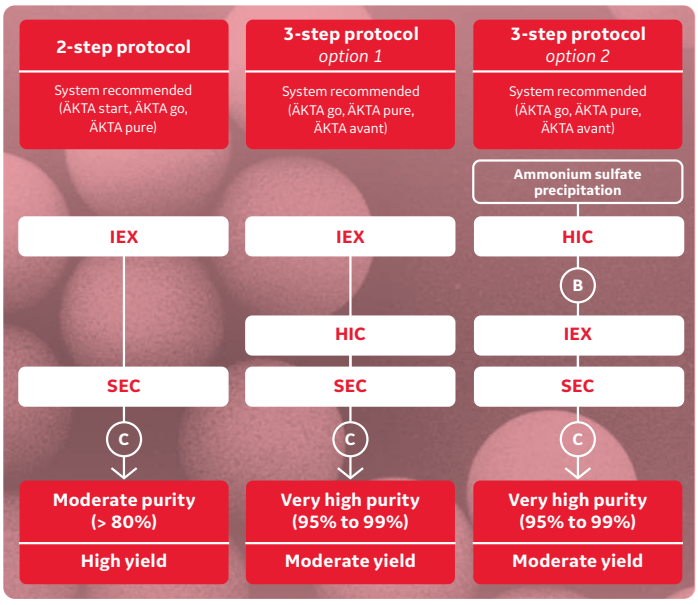
B1: Buffer exchange to neutralize low pH Ab elution buffer. B2: Buffer exchange to prepare for IEX. C: Concentration for sample volume reduction. (May also be performed before SEC.)

Which chromatography columns are recommended for each step?

	1-step protocol	2-step protocol	3-step protocol
Affinity	HiTrap Protein A HP HiTrap Protein G HP HiTrap MabSelect™ PrismA HiTrap MabSelect SuRe™	HiTrap Protein A HP HiTrap Protein G HP HiTrap MabSelect PrismA HiTrap MabSelect SuRe	HiTrap Protein A HP HiTrap Protein G HP HiTrap MabSelect PrismA HiTrap MabSelect SuRe
CIEX			HiTrap Capto S ImpAct HiScreen Capto S ImpAct
AIEX			HiTrap Capto Q HiScreen™ Capto Q
SEC		Superdex 200 Increase HiLoad Superdex 200 pg HiPrep Sephacryl S-300 HR	

Untagged protein purification

Most proteins purified in laboratory scale are affinity tagged and can therefore be purified with relative ease using affinity chromatography (AC). Sometimes the protein to be purified is untagged for the following reasons: (A) it comes from a natural source (native protein) or (B) the untagged protein is a recombinant protein that has been overexpressed without a tag, which would otherwise interfere with the protein structure or activity. Several reliable approaches to purification of untagged proteins are available.



B: Buffer exchange to prepare for IEX. C: Concentration for sample volume reduction. May also be performed before SEC.

Which chromatography columns are recommended for each step?

	1-step protocol	2-step protocol	3-step protocol
IEX or HIC	HiTrap Capto Q ImpRes HiTrap Capto SP ImpRes HiTrap Q HP HiTrap SP HP	HiTrap Capto Q ImpRes HiTrap Capto SP ImpRes HiTrap Q HP HiTrap SP HP	HiTrap Phenyl HP HiTrap Phenyl FF HiTrap HIC Selection Kit
HIC or IEX		HiTrap Phenyl HP HiTrap Phenyl FF HiTrap HIC Selection Kit	HiTrap Capto Q ImpRes HiTrap Capto SP ImpRes HiTrap Q HP HiTrap SP HP
SEC	HiLoad Superdex 30 pg HiLoad Superdex 75 pg HiLoad Superdex 200 pg HiLoad Superose 6 pg HiScale SEC columns (on demand)	HiLoad Superdex 30 pg HiLoad Superdex 75 pg HiLoad Superdex 200 pg HiLoad Superose 6 pg HiScale SEC columns (on demand)	HiLoad Superdex 30 pg HiLoad Superdex 75 pg HiLoad Superdex 200 pg HiLoad Superose 6 pg HiScale SEC columns (on demand)

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Swedish scientists make amazing spider silk from modified *E. coli* bacteria

The Stockholm-based biomaterials company is using genetically engineered bacteria and our protein purification technology to produce large quantities of the so-called spidroin proteins found in dragline silk, and then customize them for a variety of specific purposes. “Man-made spider silk can be adjusted to contain specific parts that bind to cells and promote wound healing, thereby enabling use within fields of tissue engineering, diagnostics and cell culture,” says Kristina Martinell, Spiber Technologies AB production director. “In short, it’s a tailor-made biomaterial.”

Spiber can now manufacture spider silk fiber, film, foam and even mesh. The company says that the material is as strong as mammalian tendons and remains stable at boiling temperatures of up to 267 degrees Celsius (512 Fahrenheit).

Over time, the company’s technique has evolved to keep the material soluble until it is ready to be shaped into the arrangements needed for various applications.

As a result, the range of potential products is huge. The company is working to apply spider silk in several medical fields, including cardiology, heart tissue regeneration, bone reconstruction, skin cell growth and vaccines.

Image credit: Spiber Technologies

*Read more **here**.*

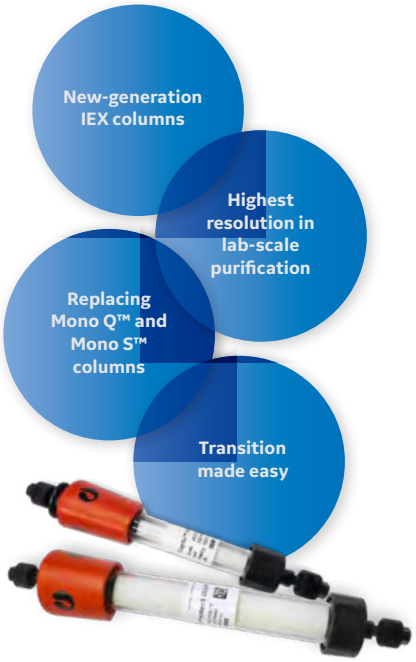
Featured products

Capto HiRes – When the highest resolution in IEX matters!

In many research areas, for example in structural biology using X-ray crystallography or cryo-electron microscopy (cryo-EM), obtaining homogeneous size and charge of biomolecules is crucial for the elucidation of their structures. High-resolution separation of samples based on their charge properties is essential to secure sample charge homogeneity and success of the study.

Capto Q HiRes and Capto S HiRes replace MonoBeads columns

A separation that worked on a Mono Q or Mono S column may be performed on a Capto HiRes Q or Capto HiRes S column with little modification or optimization. Similar resin selectivity and slightly improved resolution can be expected with the Capto HiRes columns while using the same experimental conditions. The similar selectivity of the two columns ensures a smooth transition even for quality control (QC) applications.



Learn more about our Capto HiRes ion exchange chromatography columns.

Click ***here*** for more information.

Ordering information

Resin	Format	Description	Volume	Pack size	Item
Ni Sepharose™ excel	Pre-packed columns	HisTrap excel 5 × 1mL	1 mL/column	1/pk	17371205
Ni Sepharose excel	Pre-packed columns	HisTrap excel 5 × 5 mL	5 mL/column	1/pk	17371206
StrepTactin Sepharose	Pre-packed columns	StrepTrap HP 5 × 1 mL	1 mL/column	1/pk	28907546
StrepTactin Sepharose	Pre-packed columns	StrepTrap HP 5 × 5 mL	5 mL/column	1/pk	28907548
MabSelect SuRe	Pre-packed columns	HiTrap MabSelect Sure, 1 × 1 mL	1 mL/column	1/pk	29049104
MabSelect SuRe	Pre-packed columns	HiTrap MabSelect Sure, 1 × 5 mL	5 mL/column	1/pk	11003494
MabSelect PrismA	Pre-packed columns	HiTrap MabSelect PrismA 1 × 1 mL	1 mL/column	1/pk	17549851
Capto Q ImpRes	Pre-packed columns	HiTrap Capto Q ImpRes 5 × 1 mL	1 mL/column	1/pk	17547051
Capto Q ImpRes	Pre-packed columns	HiTrap Capto Q ImpRes 5 × 5 mL	5 mL/column	1/pk	17547055
Capto SP ImpRes	Pre-packed columns	HiTrap Capto SP ImpRes 5 × 1 mL	1 mL/column	1/pk	17546851
Capto SP ImpRes	Pre-packed columns	HiTrap Capto SP ImpRes 5 × 5 mL	5 mL/column	1/pk	17546855
Capto S ImpAct	Pre-packed columns	HiTrap Capto S ImpAct 5 × 1 mL	1 mL/column	1/pk	17371751
Capto S ImpAct	Pre-packed columns	HiTrap Capto S ImpAct 5 × 5 mL	5 mL/column	1/pk	17371755
Capto S ImpAct	Pre-packed columns	HiScreen Capto S ImpAct	4.7 mL/column	1/pk	17371747
HIC Resin	Pre-packed columns	HiTrap HIC Selection Kit, 7 × 1 mL	1 mL/column	1/pk	28411007
Capto HIC	Pre-packed columns	HiTrap Capto HIC Selection Kit 5 × 1 mL	1 mL/column	1/pk	29321087
Superdex 75 prep grade	Pre-packed columns	HiLoad 16/600 Superdex 75 pg	320 mL/column	1/pk	28989333
Superdex 200 prep grade	Pre-packed columns	HiLoad 16/600 Superdex 200 pg	120 mL/column	1/pk	28989335
Superose 6 prep grade	Pre-packed columns	HiLoad 16/600 Superose 6 pg	120 mL/column	1/pk	29323952
Superdex 30 Increase	Pre-packed columns	Superdex 30 Increase 10/300 GL	24 mL/column	1/pk	29219757
Superdex 75 Increase	Pre-packed columns	Superdex 75 Increase 10/300 GL	24 mL/column	1/pk	29148721
Superdex 200 Increase	Pre-packed columns	Superdex 200 Increase 10/300 GL	24 mL/column	1/pk	28990944
Capto HiRes Q	Pre-packed columns	Capto HiRes Q 5/50	1 mL/column	1/pk	29275878
Capto HiRes S	Pre-packed columns	Capto HiRes S 5/50	1 mL/column	1/pk	29275877
Sephadex G-25	Pre-packed columns	HiTrap Desalting , 5 × 5 mL	5 mL/column	1/pk	17140801
Sephadex G-25	Pre-packed columns	HiPrep 26/10 Desalting	53 mL/column	1/pk	17508701

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Stripping and reprobing Western blot membrane: problems and solutions

Multiple uses of your blotting membrane can be especially useful if your proteins of interest are only available in limited quantities.

Stripping the membrane involves harsh conditions to disrupt the interaction between the membrane-bound protein and the primary antibody. This process enables reprobing with a new primary antibody for further protein identification. Careful consideration of the stripping conditions can help minimize the risk of protein loss from the membrane. These considerations include using combinations of detergents, reducing agents, heat, and high or low pH.

There are a few things to bear in mind once you know you are going to reuse a membrane. Your target protein abundance and antibody affinities are two points to consider. These properties influence your membrane stripping effectiveness, and which antibody you use first.

Strategy 1 – Problem: you have two proteins of similar abundance and two antibodies of similar affinity. Solution: you can detect either protein first, strip the membrane, and then detect the remaining protein.

Strategy 2 – Problem: you have two proteins of similar abundance and two antibodies of unequal affinity. Solution: detect the protein with the lowest affinity antibody first, strip the membrane, and then detect the protein with the highest affinity antibody.

Strategy 3 – Problem: you have two proteins of different abundances (one high and one low) and antibodies of equal affinity. Solution: detect the low-abundance protein first, strip the membrane, and then detect the high-abundance protein.

Strategy 4 – Problem: you have two proteins of different abundances (one high and one low) and antibodies of unequal affinity. Solution: detect the low-abundance protein first, strip the membrane, and then detect the high-abundance protein.

When using enhanced chemiluminescence (ECL) detection for a Western blot, a sequential labeling method is available for quick detection of a second protein on a single membrane.

Alternative methods of detecting additional proteins

- Sequential labeling with ECL detection
- Multiplex detection

Labeling and detection of the first protein is performed as normal using ECL. The horseradish peroxidase (HRP) is then inactivated (quenched) using hydrogen peroxide (H_2O_2) and the membrane is washed. As a result, the second protein can be labeled with a different antibody for detection without any interference.

Multiplex detection - To avoid stripping and reprobing altogether, multifluorescence (multiplex) detection can be used to detect multiple proteins on the same membrane. In this technique, secondary antibodies labeled with fluorophores enable simultaneous detection of more than one protein.

*Download handbook **here**.*

*For more information please visit **eu.fishersci.com***

Featured products

Amersham™ ECL™ detection reagents

ECL based on horseradish peroxidase (HRP)-conjugated secondary antibodies has become the most commonly used detection method for Western blotting. It is a sensitive detection method, where the light emission is proportional to protein quantity.

Minute quantities of proteins can be detected and quantitated.

- Longer shelf life: up to 18 month shelf life on ECL Select™ and Prime products.
- Stability: ECL Select and ECL Prime products are stable and stored at room temperature.

Amersham Hyperfilm™ ECL detection film

This is a sensitive film for the detection of chemiluminescent signals in Western blotting assays.

- Clear background for excellent contrast and band visibility.
- Publication-quality images.
- Learn more here: gelifesciences.com/wbfaq.

Amersham Western blotting membranes

We offer a broad selection of nitrocellulose (NC) and polyvinylidene difluoride (PVDF) Western blotting membranes, with pore size ranges to suit your application requirements.

- Optimized for chemiluminescent and fluorescent detection.
- Excellent protein binding capacity over a wide size range.
- New larger pack sizes reduce your price per blot by up to 30%.

CyDye™ labeling reagents

CyDye Fluors are fluorescent dyes used in applications such as microarray analysis, FISH, 2-D DIGE, immunoprecipitation, and blotting.

Dyes are packaged in premixed amounts and foil-sealed to ensure consistent labelings.

Amersham ECL Rainbow™ molecular weight markers:

Accurate size determination of your protein on gels and blots. Download a brochure **here**.

Ordering information

Chemistry	Format	Description	Volume/size	Pack size	Item
Chemiluminescent	Kit	ECL Western blotting detection reagent	For 2000 cm² membrane	1/pk	RPN2209
Chemiluminescent	Kit	ECL Select Western blotting detection reagent	For 1000 cm² membrane	1/pk	RPN2235
Chemiluminescent	Kit	ECL Prime Western blotting detection reagent	For 3000 cm² membrane	1/pk	RPN2236
Chemiluminescent	Kit	QuickStain kit	1 µg/mL to 20 mg/mL	1/pk	RPN4000
Chemiluminescent	Kit	Full range Rainbow molecular weight marker	250 µL	1/pk	RPN800E
Chemiluminescent	Sheets	Amersham Hyperfilm ECL	5 × 7 inches	50/pk	28906835
Chemiluminescent	Roll	Amersham Hybond™ PVDF membrane	0.2 µm, 260 mm × 4 m	1 roll	10600021
Chemiluminescent	Roll	Amershan Protran™ supported NC membrane	0.45 µm, 300 mm × 4 m	1 roll	10600016
Fluorescent labeling	Kit	Amersham CyDye Value Packs - Cy™5 Mono - NHS Ester	10 mg	1/pk	PA15104
Fluorescent labeling	Kit	Amersham CyDye Value Packs - Cy7 Mono - NHS Ester	10 mg	1/pk	PA17104

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Sera-Mag Nucleic acid sample and library preparation

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- Sera-Mag Streptavidin
- Sera-Mag Carboxyl
- SeraSil-Mag
- TempliPhi™
- GenomiPhi™
- ExoProStar™
- PuReTaq and Hot Start RTG PCR beads
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- Nucleon

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HyClone Cell culture media and sera

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- RPMI
- Serum
- Reagents and buffers
- Process water
- Classical media

Products for cell separation and isolation:

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Sample prep

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- SPARTAN™ syringe filters
- GD/X syringe filters
- Mini-UniPrep filter vials
- 934-AH™ RTU
- GF/C™ RTU
- Polycap SPF
- Vacu-guard
- Benchkote
- Custom designed filtration
- Custom folded filter papers (cone/pyramid)

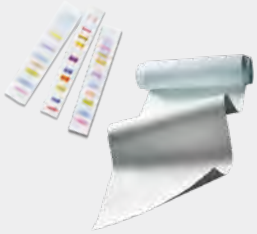
Purification

ÄKTA Chromatography columns, resins and systems

Key products:

- HiTrap columns
- HisTrap columns
- PD-10 desalting columns
- HiLoad columns
- Superdex Increase columns
- Ni Sepharose resin
- MabSelect PrismA resin
- Protein G Sepharose resin
- Capto Q resin
- Capto S ImpAct resin
- Capto ImpRes resin

Analysis



Amersham Systems, membranes, films and reagents

Key products:

- ECL detection reagents
- Rainbow markers
- Amersham ECL Gels
- CyDye labelling kits
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