

Lab recharge 2020

Life science research solutions for academia

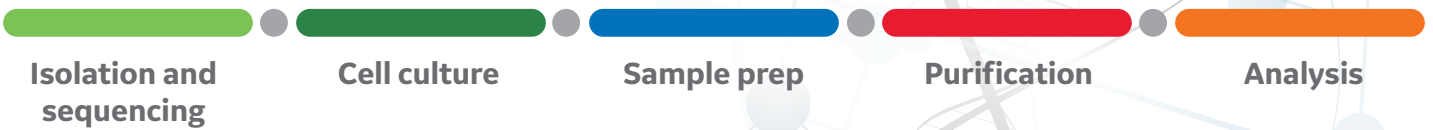
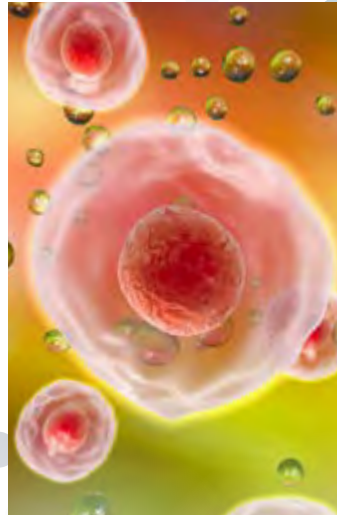
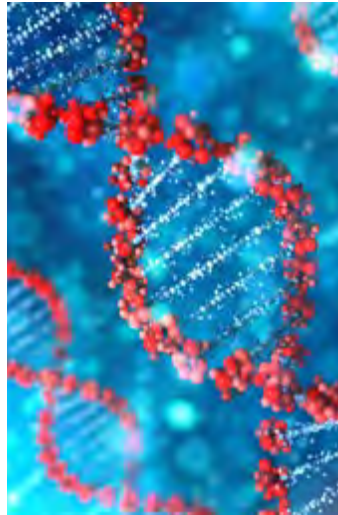
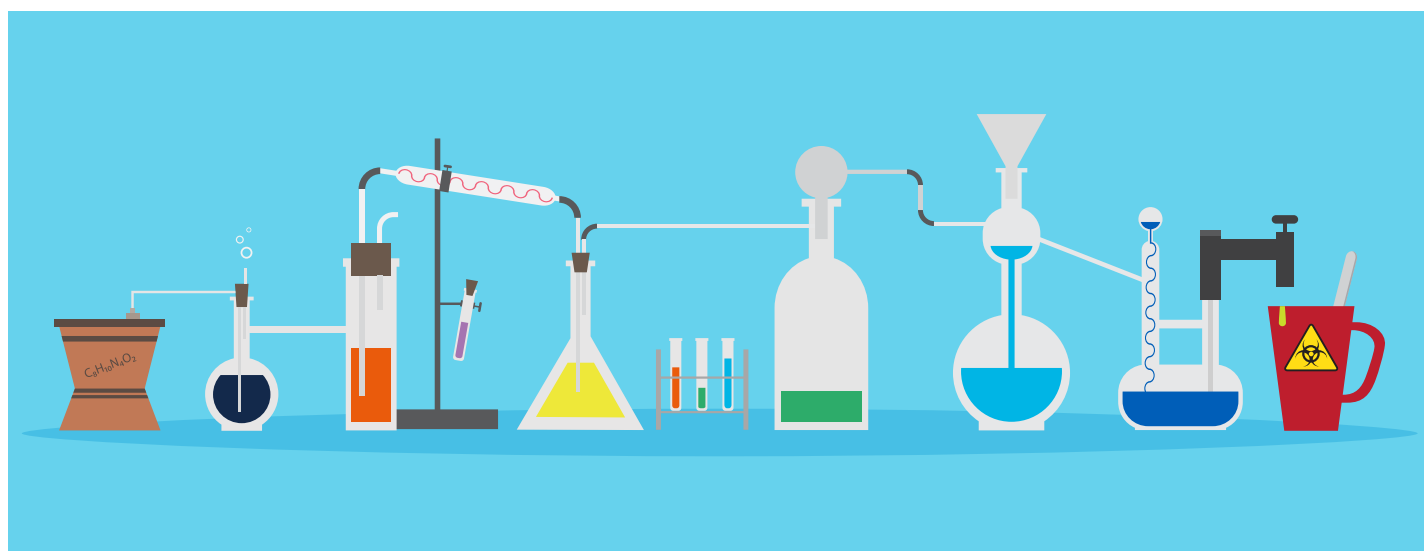


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Tools to support your science

Handbooks

Click here to request principles and methodology handbooks



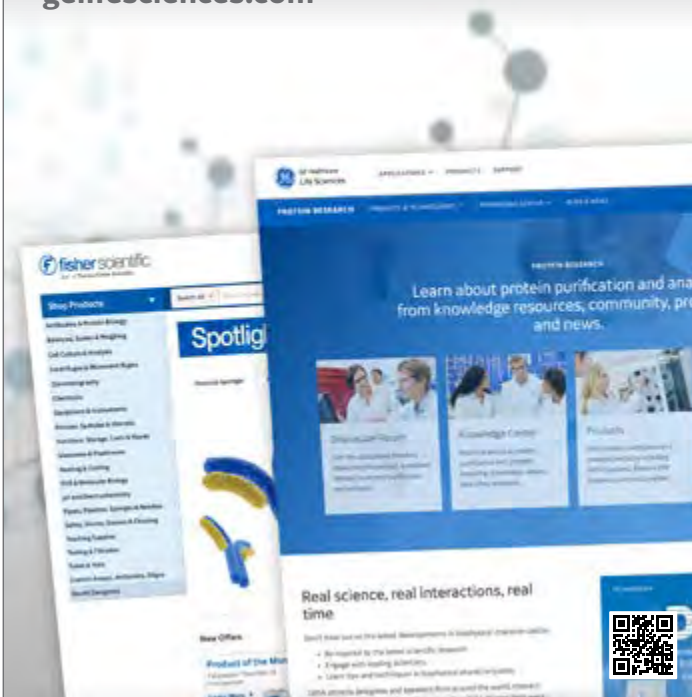
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Case study: Sera-Mag™ Select in a targeted resequencing approach

Dr. rer. nat. Stefanie Stepanow, an expert in next generation sequencing (NGS) in a molecular diagnostic laboratory in Cologne, is responsible for an NGS core facility and the establishment and validation of new assays into the routine. She tested Sera-Mag Select in a targeted resequencing approach in August, 2018. Sera-Mag Select is a ready-to-use reagent based on our Sera-Mag Carboxyl SpeedBead and the well-known solid phase reversible immobilization technology. It provides optimal binding characteristics for both polymerase chain reaction (PCR) clean-up and DNA fragment size selection for NGS library preparation.

DNA

Genomic DNA from four reference samples purchased from Coriell Institute for Medical Research were used:

- NA12878 (HG001)
- NA24385 (HG002)
- NA24149 (HG003)
- NA24143 (HG004)

Published high confidence data sets are available for these samples, which can be used for the assessment of accuracy, sensitivity and specificity of a next-generation targeted sequencing approach.

Reagents for library preparation

The libraries were constructed by using NEBNext™ Ultra (New England Biolabs Inc.) library preparation kit for the

Illumina™ platform. Then the xGen™ Inherited Disease Panel (Integrated DNA Technologies), which enables deeper sequencing of genomic regions containing genes and SNPs associated with inherited diseases, was utilized to explore the presence of inherited diseases (*protocol can be found here*).

Kit	Component	Vendor
NEBNext Ultra DNA Library Prep Kit for Illumina	E7370	NEB
NEBNext Multiplex Oligos for Illumina (Dual Index Primer Set 1)	E7600S	NEB
xGen Inherited Disease Panel v1.0 (16x)	1016352	IDT
xGen Hybridization and Wash Kit (16x)	1080577	IDT

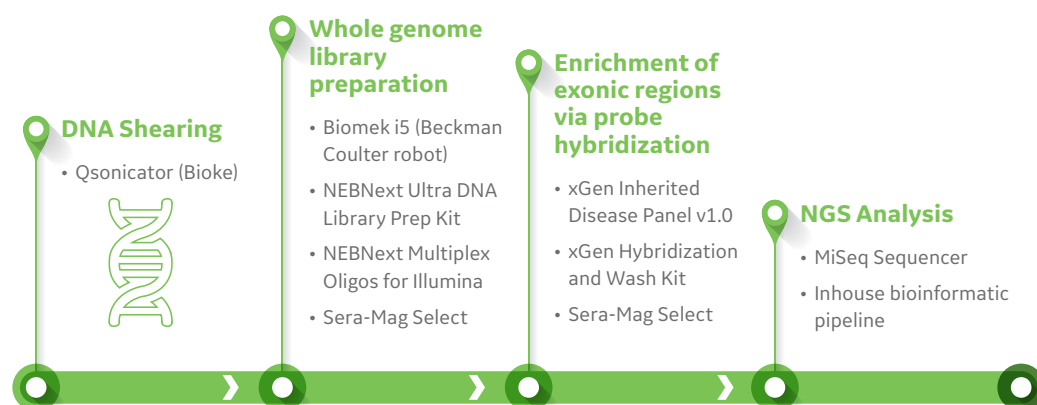
Sera-Mag Select magnetic particles were used for size selection and PCR clean-up during all of the steps, following the standard protocols for these products.

Kit	Component	Vendor
Sera-Mag Select 60 mL	29343052	GE Healthcare

Sequencing

A MiSeq™ Reagent v3 600 cycle kit (Illumina) was used to allow for paired end sequencing and read lengths up to 2 × 300 bp.

Kit	Component	Vendor
MiSeq Reagent Kit v3, 600 cycles	MS-102-3003	Illumina



Standard NGS workflow showing the products used in the targeted sequencing approach.



Read full case study [here](#).



Featured products

Sera-Xtracta™ Cell-Free DNA Kit

NEW PRODUCT

Designed to select for small-fragment cfDNA whilst minimizing genomic DNA contamination, Sera-Xtracta Cell-Free DNA Kit offers rapid extraction and purification of cell-free DNA from plasma. Its high yield and sensitivity make the kit ideal for applications such as cancer diagnosis and monitoring where sample is precious. Compatible with molecular biology techniques, including next generation sequencing (NGS), qPCR, ddPCR, BEAMing and other amplification and genotyping applications.



Sera-Xtracta Genomic DNA Kit

NEW PRODUCT

High performance isolation kit for whole blood that delivers the best combination of yield, purity and extraction of long DNA fragments with a simplified protocol. The rapid protocols designed to minimize shearing, result in high quality, intact genomic DNA compatible with molecular biology techniques, including next generation sequencing (NGS), PCR amplification and genotyping applications.



Sera-Mag Select

NEW PRODUCT

Sera-Mag Select PCR clean-up and size selection reagent combines the exceptional binding characteristics of Sera-Mag Carboxyl Speedbeads with an optimized binding solution in a ready-to-use formulation. The DNA size range isolated from the process can be tailored to suit the user's end requirements by adjusting the amount of reagent that is mixed with a fixed volume of sample.



NGS guide

We are advancing the simplification and accessibility of the NGS technologies for day-to-day research, clinical diagnostics and much more. Find out about the developing technologies and the products available to support your workflows.

Download [here](#).



Ordering information

Chemistry	Format	Description	Volume	Pack size	Item
Phi29 DNA polymerase	Kit	TempliPhi™ 100 Amplification Kit	100 rxns	1/pk	25640010
Phi29 DNA polymerase	Kit	TempliPhi 500 Amplification Kit	500 rxns	1/pk	25640050
Enzymatic PCR clean up technology	Kit	ExoProStar™-1 Step (100 RCN)	100 rxns	1/pk	US77702
Enzymatic PCR clean up technology	Kit	ExoProStar-1 Step (500 RCN)	500 rxns	1/pk	US77705
Enzymatic PCR clean up technology	Kit	ExoProStar S 100	100 rxns	1/pk	US79010
Enzymatic PCR clean up technology	Kit	ExoProStar S 500	500 rxns	1/pk	US79050
Sera-Mag Carboxyl Speedbeads	Kit	Sera-Mag Select NEW	5 mL	1/pk	29343045
Sera-Mag Carboxyl Speedbeads	Kit	Sera-Mag Select NEW	60 mL	1/pk	29343052
Sera-Mag Carboxyl Speedbeads	Kit	Sera-Mag Select NEW	450 mL	1/pk	29343057
Magnetic beads	Kit	Sera-Xtracta Cell-Free DNA Kit NEW	96 rxns	1/pk	29437807
Magnetic beads	Kit	Sera-Xtracta Genomic DNA Kit NEW	48 rxns	1/pk	29429149



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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.



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 <i>in vitro</i> 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
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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Glutamine stability in cell culture media

Glutamine is an essential amino acid for most cell lines. When stored as a dry powder or frozen solution, glutamine is relatively stable, but decomposes in aqueous solutions. The rate of decomposition depends strongly on the solution temperature. This study describes the stability of glutamine in cell culture media at different storage temperatures, and the effects of glutamine decomposition on cell growth.

Introduction

Since it was first isolated in 1932, glutamine has been the key to many aspects of mammalian cell culture. Not only does this amino acid stimulate cell growth and antibody production, it is also a major energy source in cell culture. The consumption of glutamine by cells in culture produces ammonia and pyrrolidone carboxylic acid. As the ammonia from glutamine metabolism accumulates, it inhibits cellular metabolism. Ammonia also appears in medium during storage due to the spontaneous decomposition of glutamine. Thus, the proper storage of cell culture medium is critical.

This study examines the decomposition of glutamine over time at different temperatures and the resulting effects on cell culture. Daily samples were analyzed for glutamine and ammonia levels. Growth studies were conducted using the following cell lines: SP20, AIF, NSO, CHO, VERO, BHK-21, and MRC-5. Results clearly demonstrate the instability of glutamine in media at different storage temperatures and the effects of the resulting ammonia on cell growth. Proper storage and monitoring of glutamine containing media, or the option of purchasing media without glutamine, are also emphasized.

Material and methods

Stability study

The stability study to examine the degradation of glutamine in cell culture medium was performed at common laboratory temperatures. HyClone™ DMEM/High Glucose without L-glutamine was supplemented by adding L-glutamine solution to a concentration of approximately 4 mmol/L. Two bottles were placed at each of the three temperature conditions: 2°C–8°C (laboratory cooler), 22°C (laboratory bench), and 37°C (cell culture incubator). Samples were periodically

taken and analyzed using a BioProfile™ chemical analyzer (Nova Biomedical) for glutamine and ammonium levels.

Cell growth was compared in fresh medium and medium stored 30 days at 2°C–8°C, 22°C, and 37°C using seven cell lines. One bottle, at each of the three conditions, was supplemented with 10% Dialyzed FBS and analyzed to determine a base line for glutamine and ammonium levels.

Growth studies

Two types of growth studies were performed using the seven cell lines in control medium and medium that had been stored at the three experimental conditions for 30 days:

1. Multiple passage studies were conducted by averaging cell yields from three to four day cultures.
2. Growth curve studies were conducted by measuring cell counts and medium profiles daily.

Additional growth studies were performed to differentiate effects caused by accumulation of the ammonium ion from effects caused by the depletion of L-glutamine. Medium stored at 37°C for 30 days was supplemented with L-glutamine back to the initial 4 mmol/L.

Ammonia growth study

To further investigate the effects of ammonium accumulation and L-glutamine depletion, an additional growth study was performed. Fresh bottles of DMEM/High Glucose without L-glutamine were supplemented with 10% Dialyzed FBS and 4 mmol/L L-glutamine. The medium was divided into six 250 mL samples. The samples were spiked with an ammonium chloride solution to create six conditions: 0, 2, 4, 6, 8, and 10 mmol/L ammonium. AIF, SP20, BHK21, and CHO cell lines were grown over four passages.

Results

Stability study

Seven common cell lines were grown over four passages in DMEM supplemented with 10% Dialyzed FBS.

Growth studies

Initial readings of glutamine and ammonium levels were performed on day 30. The growth studies clearly illustrate the effects of medium storage temperatures on cell growth. Cells grew relatively well in medium stored at 2°C–8°C, marginally well in medium stored at 22°C, and poorly in medium stored at 37°C. The additional growth studies show that re-supplementation of glutamine enhances cell growth in medium stored 30 days at 37°C. However, growth was only about 50% or less of the control in some cell lines. This study also indicates that some cell lines may be able to grow well in moderate ammonia levels.

Ammonia growth study

The data from the ammonia growth study indicate that ammonia has a significant effect on cell growth. All four studied cell lines show significantly decreased growth when ammonium concentrations are 4 mmol/L or greater.

Conclusions

Glutamine decomposition in cell culture medium is properly of concern to many researchers. The breakdown of this key amino acid has negative effects on cell culture. This study clearly demonstrates the relative stability of glutamine in medium stored at different temperatures and the effects of ammonia buildup on the growth of some commonly used cell lines.

We recommend storing liquid medium containing glutamine at 2°C–8°C, or purchasing medium without glutamine and adding it at the time of use.

Featured products

Liquid Cell Boost bottles

The supplement is available as a ready to use liquid and in dry powder format for research, process development and manufacturing. The liquid feed supplement meets testing specifications and is stable up to 3 months at 2°C to 8°C.

Research classical media small volume

Cell culture media include inorganic salts, amino acids, carbohydrates, vitamins, and other nutrients capable of sustaining cell growth. HyClone quality media provide consistent cell culture performance in basic research and biopharmaceutical manufacturing.

Dissociation and cryopreservation

Trypsin cell detachment solution: the HyClone portfolio includes various concentrations of trypsin protease.

- Derived from porcine pancreas
- Gamma irradiated prior to hydration and filling
- Formulated without calcium and magnesium

HyCryo-STEM maintains differentiation potential and minimizes spontaneous differentiation of stem cells.

- Designed to freeze cells sensitive to the cryopreservation process
- Helps maintain cell stemness, providing healthy and stable stocks of stem cells for downstream applications
- Provided at a 2X concentration for addition to cells suspended in their own conditioned growth medium to minimize osmotic shock during cryopreservation
- Chemically defined and serum-free to ensure lot-to-lot consistency



Try Whatman™ syringe filters to prepare your sample.

Click [here](#) for further information.



Ordering information

Product type	Format	Description	Volume	Pack size	Item
Classical Media	Bottle	DMEM with High Glucose, with 4.0 mM L-Glutamine, without Sodium Pyruvate	500 mL	1/pk	SH30022.01
Classical Media	Bottle	MEM with Earle's Balanced Salt Solution (EBSS), with 2.0 mM L-Glutamine	500 mL	1/pk	SH30024.01
Classical Media	Bottle	RPMI 1640, 1X, with 2.05 mM L-Glutamine	500 mL	1/pk	SH30027.01
Cryopreservation	Bottle	HyCryo 2x Cryopreservation Media	100 mL	1/pk	SR30001.02
Cryopreservation	Bottle	HyCryo-STEM 2x Cryopreservation Media	100 mL	1/pk	SR30002.02
Cell detachment	Bottle	Trypsin 0.25% (1X) Solution, with 2.5 g Porcine Trypsin (1:250)/L in HBSS, without Calcium and Magnesium, with 0.1% EDTA	100 mL	1/pk	SV30031.01
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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Improve lab efficiency through better filtration

Do you consider particle retention, loading capacity, and liquid flow rate when choosing a filter or device? Perhaps there is a better filter out there for your application. Or perhaps your analysis might be easier, quicker, or produce results that are more consistent if you switched your filter to a different grade.

Here are three key characteristics to consider when identifying the right filter.

1. Particle retention

For cellulose and glass microfibre papers, it is expressed as a “nominal retention rating”, and quoted at 98% efficiency to allow for secondary filtration effects. For membrane filters with defined pore sizes, it is an absolute retention rating.

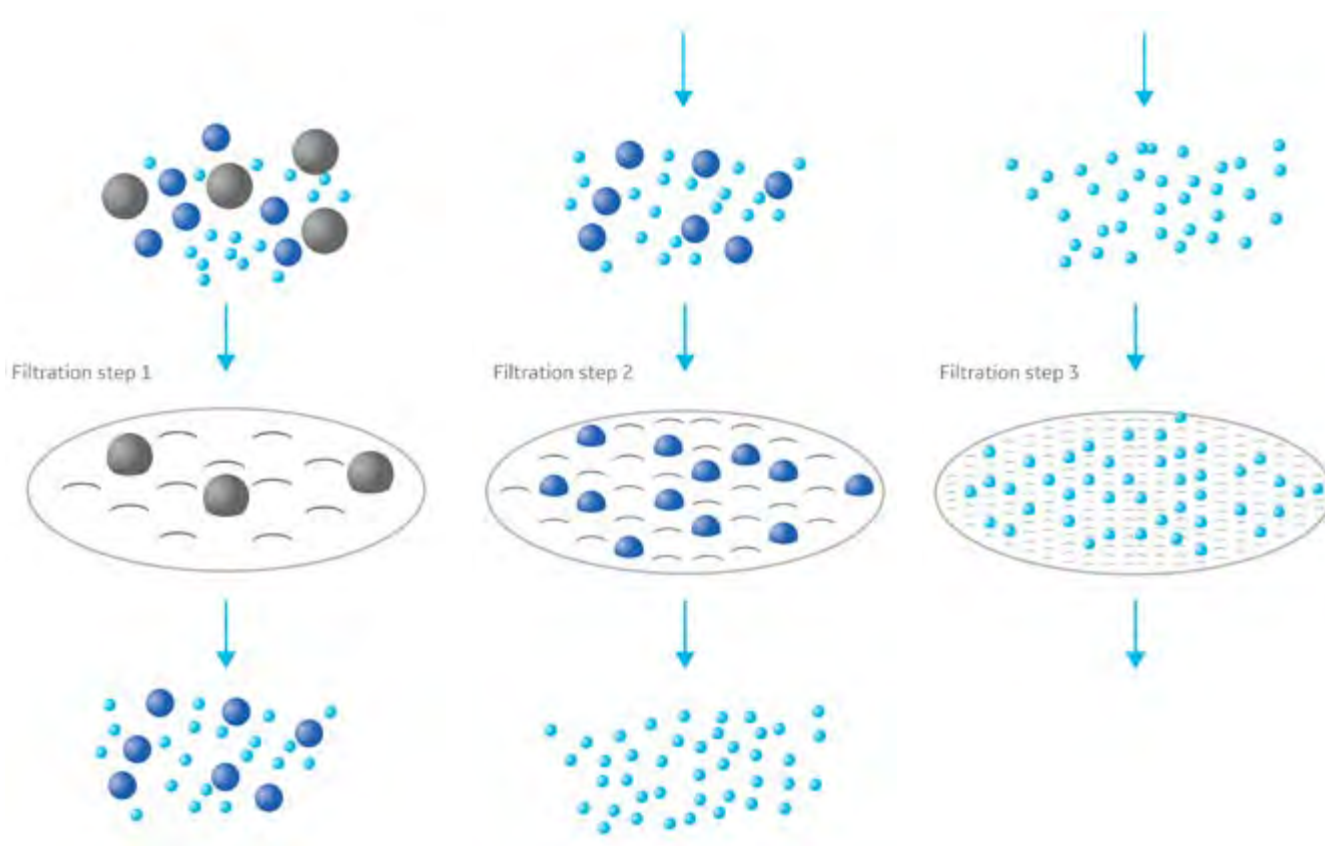
2. Loading capacity

Filters with the highest loading capacities are chemically treated

and are more expensive than their untreated counterparts. Treatment might also interfere with analysis. This can happen either through chemical interaction with the sample or by increasing the time to results due to a slower flow rate than that of an untreated filter. By knowing the weight of filtrate that you want to retain on the filter, you can choose a filter that will safely accommodate your needs without the downsides of a filter that is more complex than is needed.

3. Liquid flow rate

The flow rate describes the speed at which a liquid flows through the filter. In practice, this is dependent on several factors that will often be specific to the solid/liquid being filtered. But, for comparison purposes, a typical water flow rate is measured and provided for each grade under gravity and normalized to a certain diameter.



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.



Featured products

Whatman Uniflo™ syringe filters

Disposable filter units designed to provide clean filtrate from small volumes up to 100 mL. Available in a variety of membrane choices with a polypropylene overmold housing. Whatman Uniflo syringe filters are available with:

- 13 or 25 mm diameters
- 0.2 µm or 0.45 µm pore sizes
- Sterile or non-sterile options
- Individual printing on the filter for easy identification
- Bench-top space saving packaging
- Bulk pack sizes available
- **New filter media available soon:** hydrophilic-PTFE (H-PTFE) can be used for both aqueous and aggressive organic solvents



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



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.

- Compatible with most major autosamplers for high throughput analysis
- All-in-one filtration device for quick and cost-effective sample processing



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
RC	Non sterile	Mini UniPrep syringeless filter	0.2 µm	100/pk	UN203NPERC
RC	Non sterile	Mini UniPrep syringeless filter	0.45 µm	100/pk	UN203NPURC
RC	Non sterile	Whatman GD/X syringe filter	25 mm 0.2 µm	150/pk	6887-2502
RC	Non sterile	Whatman GD/X syringe filter	25 mm 0.45 µm	150/pk	6882-2504
Nylon	Non sterile	Whatman Uniflo syringe filter	13 mm 0.45 µm	500/pk	9910-1304
PES	Non sterile	Whatman Uniflo syringe filter	25 mm 0.45 µm	500/pk	9912-2504
PVDF	Sterile	Whatman Uniflo syringe filter	25 mm 0.2 µm	45/pk	9913-2502
H-PTFE	Non sterile	Whatman Uniflo syringe filter NEW	25 mm 0.2 µm	100/pk	9920-2502
Absorbent	Sheets	Benchkote™ surface protector for ÄKTA start	310 mm x 210 mm	25/pk	2300-10064



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ÄKTA start

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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

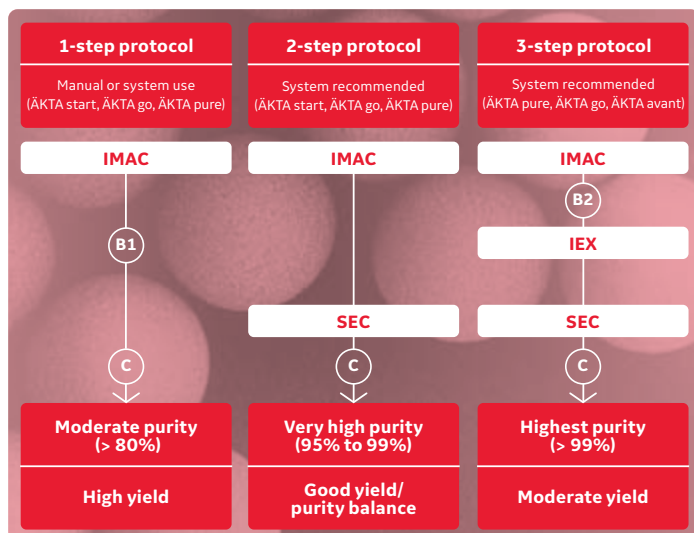
Request information [here](#).



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.



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

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.

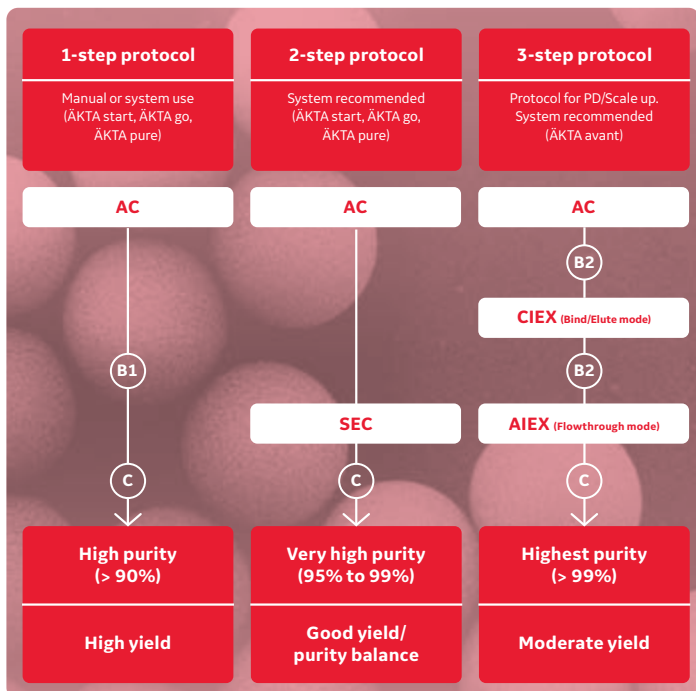


Learn more about protein purification protocols in our **Strategies for Protein Purification handbook**. Download handbook [here](#).



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.



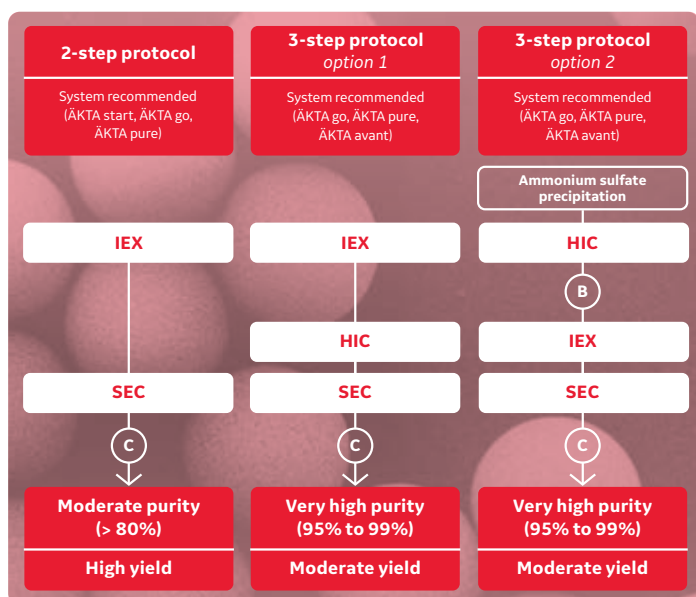
B1: Buffer exchange to neutralize low pH Ab elution buffer. B2: Buffer exchange to prepare for IEX. C: Concentration for sample volume reduction. (May also be performed before SEC.)

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’s production director. “In short, it’s a tailor-made biomaterial.”

Spiber Technologies AB 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.



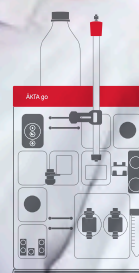
Image credit: Spiber Technologies

Read more [here](#).



Sign up for the ÄKTA club newsletter for more insights in protein purification

Sign up [here](#).



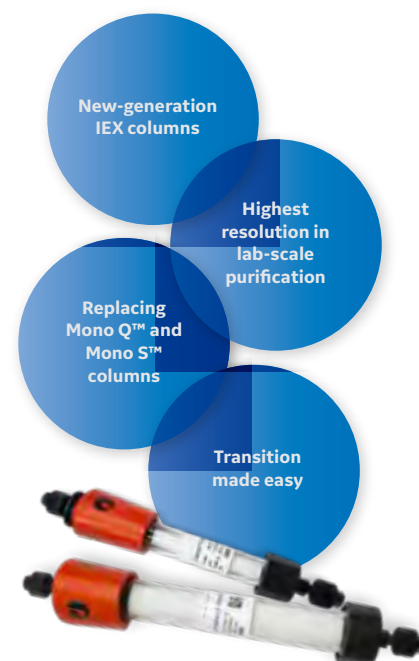
Featured products

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
—	—	ÄKTA start - Main Instrument	—	1/pk	29022094
—	—	Frac 30	—	1/pk	29023051
—	—	UNICORN Start 1.1 DVD pk + Activation code	—	1/pk	29276964
Ni Sepharose 6 Fast Flow.	Pre-packed columns	HiTrap FF Crude 5 × 1 mL	1 mL/column	1/pk	11000458
Ni Sepharose HP	Pre-packed columns	HiTrap HP 5 × 1 mL	1 mL/column	1/pk	17524701
Ni Sepharose excel.	Pre-packed columns	HiTrap excel 5 × 1 mL	1 mL/column	1/pk	17371205
TALON™ Superflow™	Pre-packed columns	HiTrap TALON crude, 5 × 1 mL	1 mL/column	1/pk	28953766
Glutathione Sepharose	Pre-packed columns	GSTrap 4B, 5 × 1 mL	1 mL/column	1/pk	28401745
Dextrin Sepharose HP	Pre-packed columns	MBPTrap 1 × 5 mL	5 mL/column	1/pk	28918779
Protein A Sepharose HP	Pre-packed columns	HiTrap Protein A HP, 2 × 1 mL	1 mL/column	1/pk	17040203
Protein G Sepharose HP	Pre-packed columns	HiTrap Protein G HP, 2 × 1 mL	1 mL/column	1/pk	17040403
Capto Q ImpRes	Pre-packed columns	HiTrap Capto Q ImpRes 5 × 1 mL	1 mL/column	1/pk	17547051
Capto SP ImpRes	Pre-packed columns	HiTrap Capto SP ImpRes 5 × 1 mL	1 mL/column	1/pk	17546851
Capto S ImpAct	Pre-packed columns	HiTrap Capto S ImpAct 5 × 1 mL	1 mL/column	1/pk	17371751
HIC Resin	Pre-packed columns	HiTrap HIC Selection Kit, 7 × 1 mL	1 mL/column	1/pk	28411007
Sephacryl S-100	Pre-packed columns	HiPrep 16/60 Sephacryl S-100 HR	320 mL/column	1/pk	17116501
Sephacryl S-200	Pre-packed columns	HiPrep 16/60 Sephacryl S-200 HR	320 mL/column	1/pk	17116601
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
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



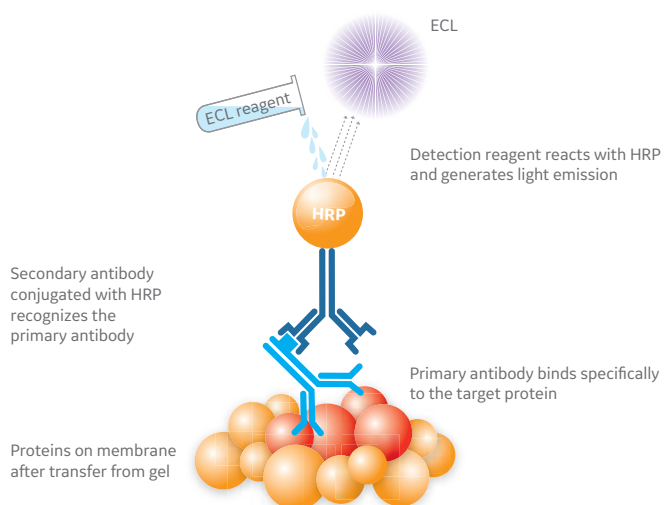
For your customized quotation and to place orders visit eu.fishersci.com

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.



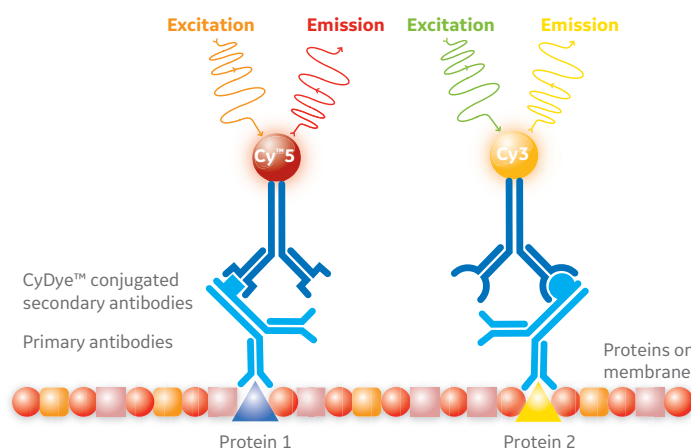
Fluorescence

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.



Featured products

Western blotting detection

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



Imaging

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%



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	Reagent	ECL Western blotting detection reagent	For 2000 cm ² membrane	1 pack	RPN2209
Chemiluminescent	Reagent	ECL Select WB detection reagent	For 1000 cm ² membrane	1 pack	RPN2235
Chemiluminescent	Reagent	ECL Prime Western blotting detection reagent	For 3000 cm ² membrane	1 pack	RPN2236
Chemiluminescent	Kit	Amersham QuickStain kit	1 µg/mL to 20 mg/mL	1 pack	RPN4000
Chemiluminescent	Kit	Full range Rainbow molecular weight marker	250 µL	1 pack	RPN800E
Chemiluminescent	Sheets	Amersham Hyperfilm ECL	5 × 7 inches	1 pack	28906835
Chemiluminescent	Roll	Amersham Hybond™ PVDF	0.2 µm 260 mm × 4 m	1 roll	10600021
Chemiluminescent	Roll	Amersham Protran™ NC	300 mm × 4m 0.45 µm	1 roll	10600016
-	Sheets	GB003 blotting paper	460 × 570 mm	50/pk	10427826



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Bringing you the established brands across the protein research workflow

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Sera-Mag Nucleic acid sample and library preparation

Key products:

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

Cell culture



HyClone Cell culture media and sera

Key products:

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

Products for cell separation and isolation:

- Percoll
- Ficoll

Sample prep



Whatman Laboratory filtration products

Key products:

- Puradisc syringe filters
- 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

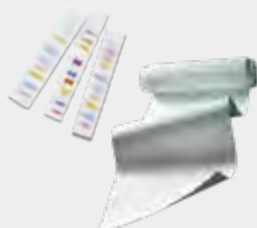


ÄKTA Chromatography columns, resins and systems

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:

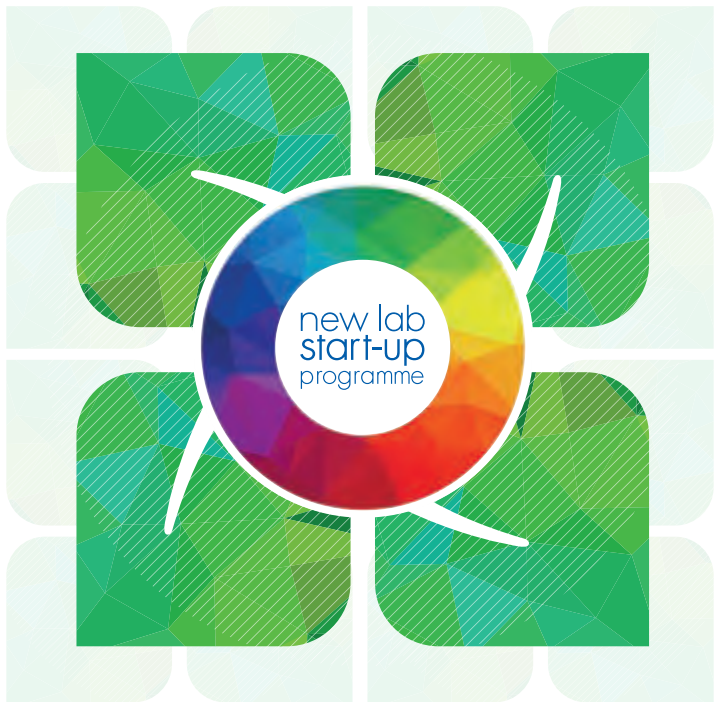
- ECL detection reagents
- Rainbow markers
- Amersham ECL Gels
- CyDye labelling kits
- NC and PVDF membranes
- Hyperfilm
- Amersham QuickStain
- PlusOne reagents
- Electrophoresis and transfer units

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Setting up a new lab?
Expanding or renovating your lab?
Relocating to new premises?
In receipt of a first time grant?

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eu.fishersci.com/go/nlsu



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We did it... Together, with our valued customers, we raised **\$121 653** for the Breast Cancer Research Foundation (BCRF) in 2019. This funds over **2500** hours of revolutionary research into the most common cancer in women worldwide, moving all of us that much closer to a cure.

For more details click [here](#).



People-Planet-Purpose

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