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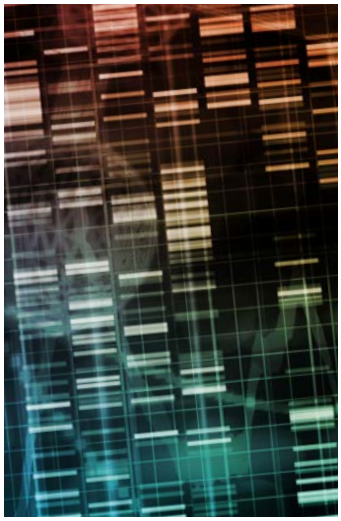
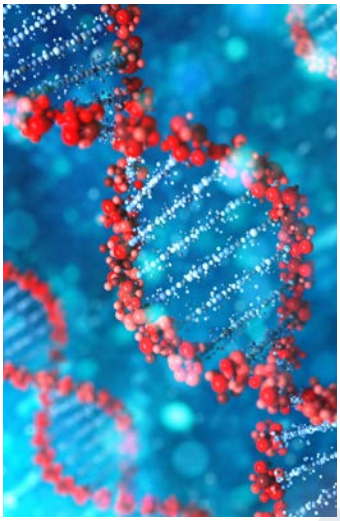
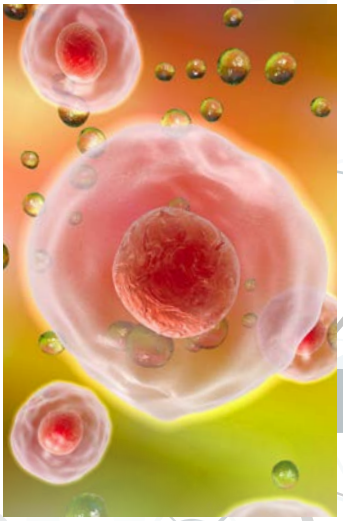
Life science research solutions for academia


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

Distributor
GE Healthcare

EUR

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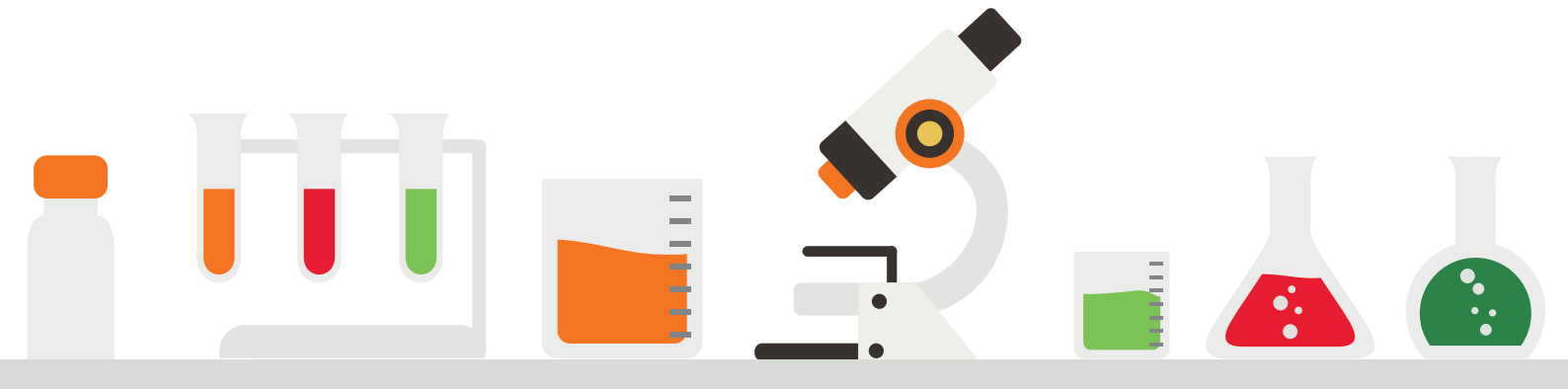

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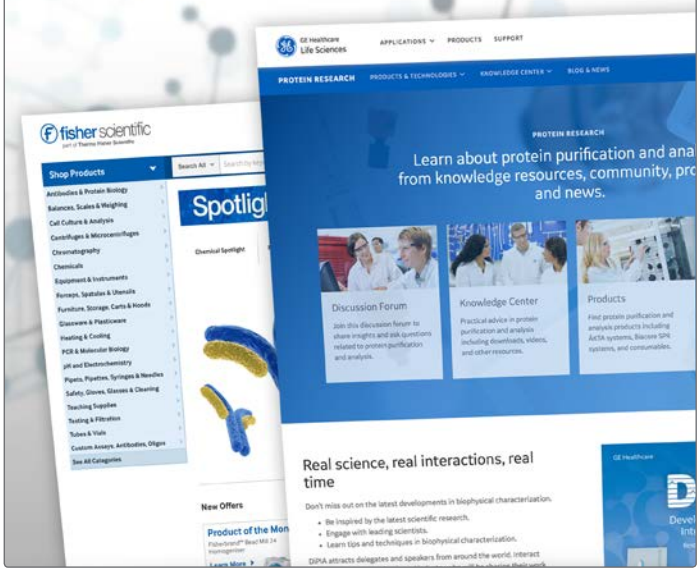

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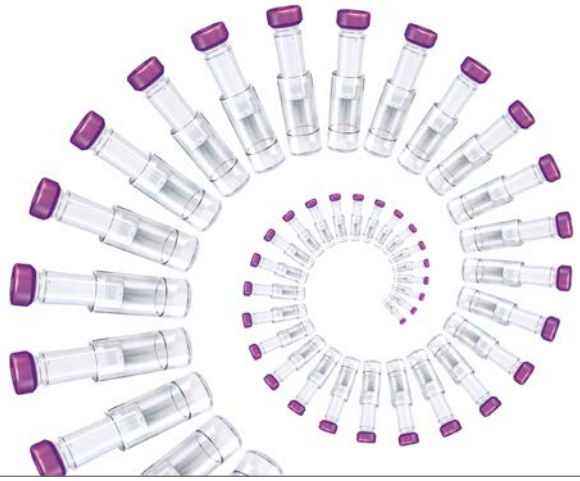

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Next-generation sequencing: Challenges (and solutions!) in sample preparation

Sample prep can have a big impact on next-generation sequencing (NGS) outcomes. Here are some simple things you can do to get your NGS off to a good start. By GE Healthcare.

How to address common challenges in next-generation sequencing sample preparation

Getting reliable data in next-generation sequencing (NGS) is all about the DNA (or RNA) you put in. How can you make sure your input DNA gives you the quality sequencing results you need?

DNA for sequencing might come from a variety of sources, including fresh tissue, formalin-fixed paraffin-embedded (FFPE) tissue, cultured cells, and liquid biopsies. Each source comes with its own challenges for maximizing the three key aspects:

- Quantity
- Integrity
- Purity

Challenge 1: Yield

Different workflows and kits vary significantly in the amount of starting material required. Your workflow might require you to use a specific type of kit, and therefore starting DNA, or vice versa.

It's important to understand which workflows and kits suit your application and the typical amounts of starting material they need. If the two don't match up, can you try another approach?

If your sample is insufficient, what can we learn from those studying at the single-cell level? Commercially available whole genome amplification (WGA) kits provide the opportunity to expand your starting material from nanograms to micrograms in a matter of hours. This technique provides improved coverage compared to PCR-based amplification and is associated with fewer amplification errors.

Your fragmentation method can also affect your final DNA yield. Physical fragmentation can result in unexpectedly small DNA fragments which can be lost, reducing the amount of DNA available for sequencing. If you have the option, enzymatic fragmentation can provide better predictability and control over fragmentation.

Challenge 2: Integrity

Having enough DNA won't make for accurate sequencing if your DNA is degraded. Degradation can affect all kinds of samples, but long-term storage and exposure to fixatives, as you might find in FFPE samples, can exacerbate the damage.

A DNA integrity number (DIN) measurement can indicate the level of DNA damage. Although not a perfect predictor of usability, or parameters such as library complexity, DIN measurement is an easy method to check DNA integrity.

Extracting a little more DNA can compensate for low quality to some extent. However, there's not much you can do about previous storage conditions, unless you can choose newer samples or those that haven't gone through such harsh processing.

If you can't acquire better samples, DNA repair might improve your outcomes. Several commercial kits can, for example, modify blocked 3' ends or fix DNA nicks. These simple repairs help make more fragments suitable for sequencing.

Challenge 3: Impurities

Okay, so you have enough DNA, and it's in pretty good condition. What else do you have in that tube?

Producing reliable results in sequencing requires samples free of proteins, organic solvents and surfactants. You might also have tissue-specific contaminants to consider.

Researchers often measure DNA purity by looking at the 260:280 nm absorbance ratio. A high-purity sample should have a 260:280 ratio of 1.8 to 2.0. Nucleic acids have an absorbance maximum at 260 nm and finding a ratio below 1.8 can indicate contamination.

As a secondary check, measure the 260:230 ratio, which will detect the presence of commonly used solvents and surfactants, such as phenol and EDTA. Values between 2.0 and 2.2 indicate high purity.

- Remove hemoglobin by preferential lysis of red blood cells early in your workflow.
- Remove heparin by washing.
- Do a phenol–chloroform extraction to reduce protein contamination.
- Use a phenol-free extraction kit to remove phenol contamination.

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

Featured products

illustra™ NAP columns

These disposable columns are prepacked with Sephadex™ G-25 DNA grade and require only gravity to run. They allow rapid and efficient purification of DNA and oligonucleotides in less than 15 min.

- Useful for small-scale purification, desalting and buffer exchange
- Available in three sizes depending on sample volume: 0.5 mL, 1 mL or 2.5 mL

illustra GenomiPhi™ V2 DNA amplification kit

Offers highly efficient and representative whole-genome amplification with 4–7 µg yield from nanogram amounts of DNA sample.

- Representative isothermal amplification of the whole genome
- No template: independent, background amplification product
- Outperforms PCR-based whole genome amplification techniques
- Quick and simple automation-friendly protocol; no thermal cycler required
- Yields high-quality DNA (e.g., for high-throughput genotyping, hybridization, and DNA archival)

illustra single cell GenomiPhi DNA amplification kit

This kit combines the novel capabilities of Phi29 DNA polymerase with an optimized formulation to amplify genomic DNA from 1–1000 cells without background interference.

- Amplifications can be completed in less than 3 hours with less than 15 min hands-on time
- Whole genome amplification of genomic DNA from as little as a single cell

Try illustra Ready-To-Go™ PCR† beads.

For robust and reproducible performance in standard PCR amplifications. Learn more **here**.

Ordering information

Chemistry	Format	Description	Volume	Pack size	Item	Price* (€)
Sephadex G-25	Column	illustra NAP-5 columns	0.5 mL	50 rxn	17085302	343,76
Sephadex G-25	Column	illustra NAP-10 columns	1 mL	50 rxn	17085402	368,94
Sephadex G-25	Column	illustra NAP-25 columns	2.5 mL	50 rxn	17085202	469,00
Sephadex G-25	Column	illustra MicroSpin™ G-25 columns	10 to 100 µL	50 rxn	27532501	201,00
Silica based	Column	illustra GFX™ PCR DNA and gel band purification kit	10 to 50 µL	100 rxn	28903470	191,34
Silica based	Column	illustra GFX PCR DNA and gel band purification kit	10 to 50 µL	250 rxn	28903471	449,00
Phi29 DNA polymerase	Kit	illustra single cell GenomiPhi DNA amplification kit	1–1000 cells	25 rxn	29108107	392,00
Phi29 DNA polymerase	Kit	illustra single cell GenomiPhi DNA amplification kit	1–1000 cells	100 rxn	29108039	1 410,00
Phi29 DNA polymerase	Kit	illustra GenomiPhi V2 DNA amplification kit	10 ng gDNA	25 rxn	25660030	171,00
Phi29 DNA polymerase	Kit	illustra GenomiPhi V2 DNA amplification kit	10 ng gDNA	100 rxn	25660031	470,00

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

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.

Request a copy of data file 29184840 AA–Glutamine stability in cell culture media–to review full results.

Featured products

HyClone South-American FBS

Research Grade Fetal Bovine Serum (FBS), South American origin is a cost-effective solution for many cell culture research applications. Typically sourced from Brazil and Uruguay, South American FBS complies with EU regulations, with strict manufacturing and quality control procedures to ensure a consistent and high-quality serum.

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

HyClone HyCryo-STEM medium is a serum-free product intended for cryopreservation and storage in biomanufacturing of stem cell lines. The medium is designed for use with neural progenitor or stem cells, embryonic stem cells (ESCs), and induced pluripotent stem (iPS) cells. This chemically defined formulation contains dimethyl sulfoxide (DMSO) and protein components to protect cells during the cryopreservation process.

Try Whatman™ syringe filters to prepare your sample.
Click **here** for further information.

Ordering information

Product type	Format	Description	Volume	Pack size	Item	Price* (€)
Fetal bovine serum	Bottle	HyClone Fetal Bovine Serum, South American Origin	500 mL	1 × 500 mL	SV30160.03	150,00
Fetal bovine serum	Bottle	HyClone Fetal Bovine Serum, South American origin, Heat Inactivated	500 mL	1 × 500 mL	SV30160.03HI	170,00
Fetal bovine serum	Bottle	HyClone Fetal Bovine Serum, South American origin, Irradiated	500 mL	1 × 500 mL	SV30160.03 IR	170,00
Classical media	Bottle	HyClone DMEM/high glucose with L-glutamine, w/o sodium pyruvate	500 mL	1 × 500 mL	SH30022.01	18,13
Classical media	Bottle	HyClone MEM with EBSS, L-glutamine	500 mL	1 × 500 mL	SH30024.01	17,77
Classical media	Bottle	HyClone RPMI 1640 media, with L-glutamine	500 mL	1 × 500 mL	SH30027.01	18,13
Cell detachment	Bottle	HyClone Trypsin	100 mL	1 × 100 mL	SV30031.01	16,30
Cryopreservation	Bottle	HyClone HyCryo	100 mL	1 × 100 mL	SR300001.02	146,29
Cryopreservation	Bottle	HyClone HyCryo-STEM	100 mL	1 × 100 mL	SR300002.02	140,19

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

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.

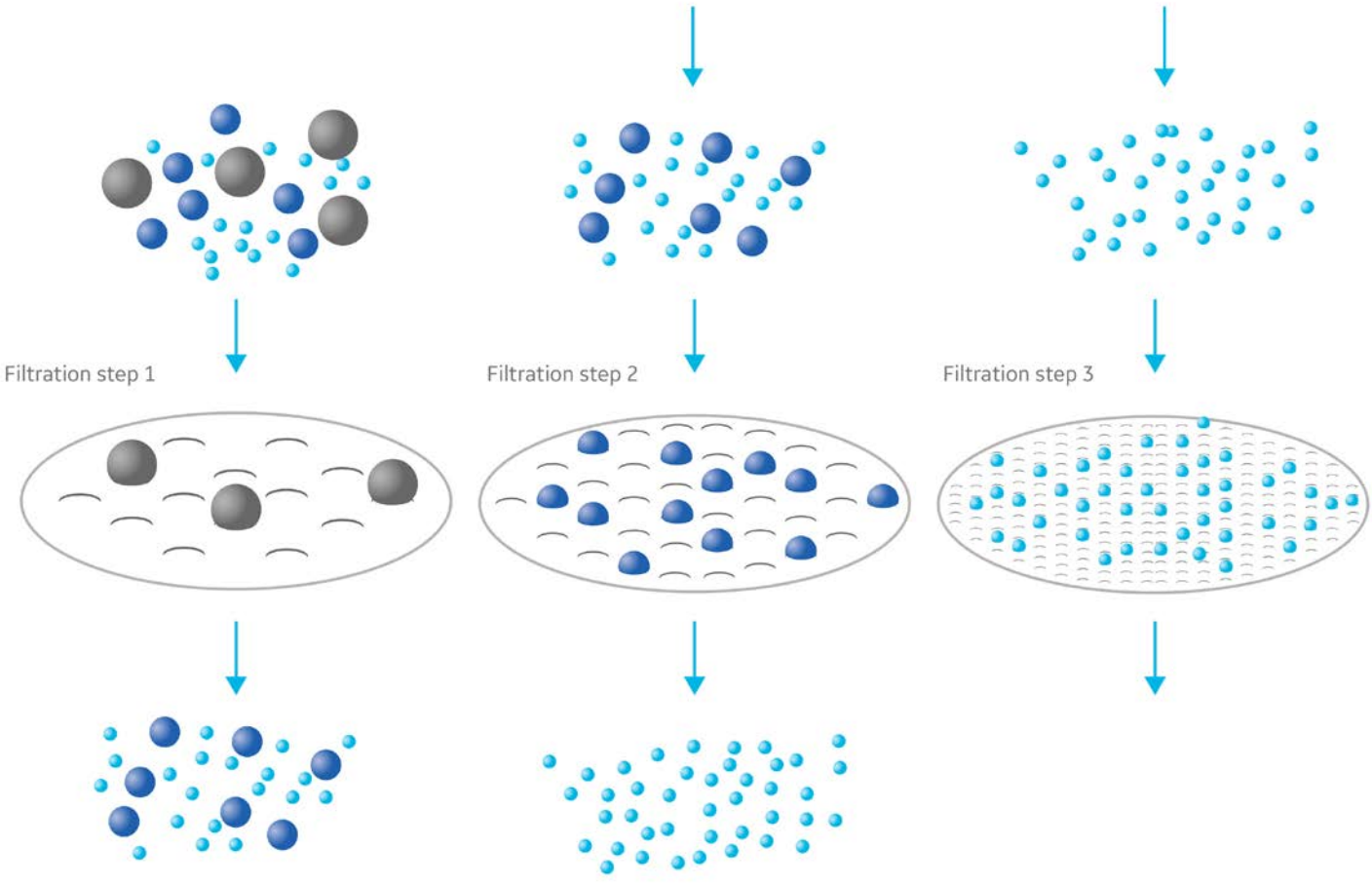
2. Loading capacity

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 App to find out if you are using the most appropriate filtration solution for your samples.



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Featured products

Whatman Uniflo™ syringe filters

NEW

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
- 0.22 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

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 new:

Protein Prep syringe filter for ÄKTA systems – **see page 11**

Ordering information

Membrane	Format	Description	Format/pore size	Pack size	Item	Price* (€)
PVDF	Non sterile	Whatman Uniflo syringe filter	25 mm 0.45 µm	500/pk	9909-2504	617,94
Nylon	Non sterile	Whatman Uniflo syringe filter	13 mm 0.45 µm	500/pk	9910-1304	660,00
PTFE	Non sterile	Whatman Uniflo syringe filter	25 mm 0.22 µm	500/pk	9911-2502	621,00
PES	Non sterile	Whatman Uniflo syringe filter	25 mm 0.45 µm	500/pk	9912-2504	621,00
PVDF	Sterile	Whatman Uniflo syringe filter	25 mm 0.45 µm	45/pk	9913-2504	117,87
PES	Sterile	Whatman Uniflo syringe filter	25 mm 0.45 µm	200/pk	9915-2504	525,00
RC	Non sterile	Whatman GD/X 25 syringe filter	25 mm 0.2 µm	150/pk	6887-2502	459,88
RC	Non sterile	Whatman GD/X 25 syringe filter	25 mm 0.45 µm	150/pk	6882-2504	511,89
RC	Non sterile	Mini-UniPrep syringeless filter	0.2 µm	100/pk	UN203NPERC	208,02
RC	Non sterile	Mini-UniPrep syringeless filter	0.45 µm	100/pk	UN203NPURC	208,02

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

New lab start-up programme

Visit the following link to find out more about the GE product offering in the Fisher new lab start-up programme:

eu.fishersci.com/go/nlsu

REQUEST DEMO

ÄKTA start

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 demo **here**.*

NEW

Sample preparation with the Protein Prep syringe filter for ÄKTA systems

Protein Prep syringe filters are ready-to-use with polycarbonate housing and a regenerated cellulose membrane that is low protein binding and broadly compatible with common solvents. Syringe filtration has been shown to reduce debris residue in the column that could otherwise impact performance and column life. In addition, the Protein Prep syringe filter is lot certified for low levels of extractable particles that might otherwise interfere with chromatograms.

Protein Prep syringe filter for ÄKTA systems

- 13 mm or 30 mm diameter
- 0.2 µm or 0.45 µm pore size

Tips for choosing the right filter

- Use 13 mm diameter filter for sample volumes < 10 mL
- Use 0.2 µm pore size filter if the particle size of the chromatography resin is < 30 µm

Protein Prep syringe filter for ÄKTA systems

*Request your sample **here**.*

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

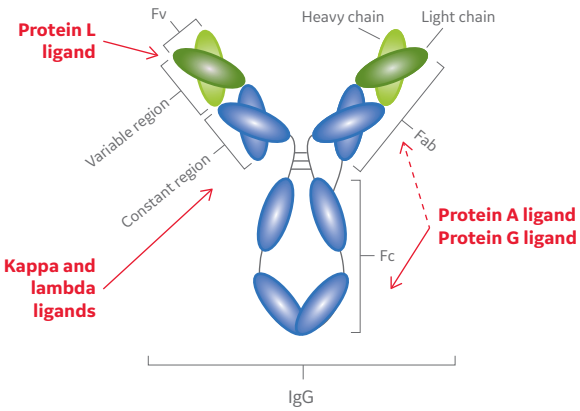
Use of affinity chromatography for antibody purification

How does antibody purification work?

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

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

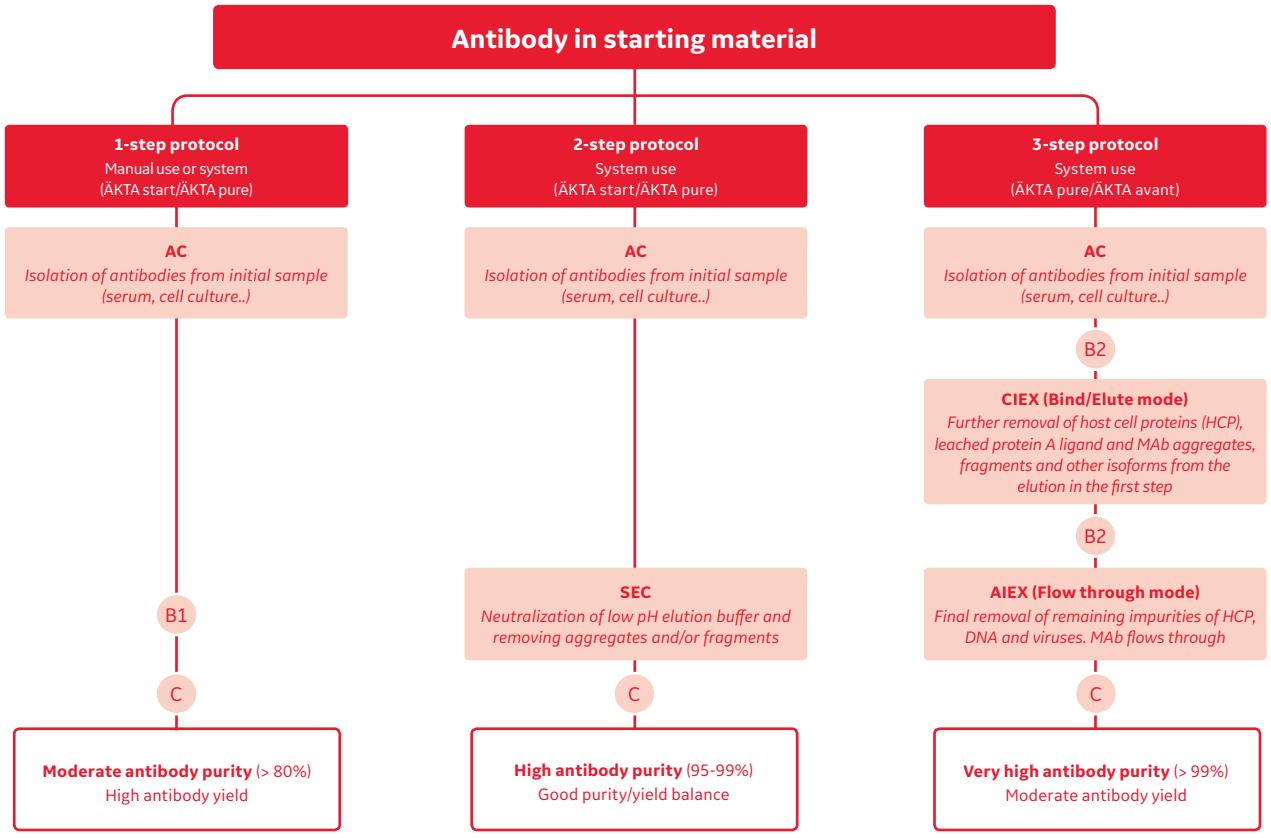
IgG, which is by far the most common immunoglobulin, is commonly purified with protein G and protein A, both of which have a strong affinity to the Fc region of IgG. Protein L has a strong affinity to the variable region of kappa light chains.



What do antibody purification schemes look like?

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

The 2-step protocol is the recommended best choice for research use. The 3-step protocol considers upscaling or process development needs. SEC is not used as a final step to remove aggregates, fragment or other impurities due to the limitations of sample volume. Instead, a combination of IEX steps is used.



C Optional concentration B1 Buffer exchange to neutralize low pH elution buffer B2 Optional buffer exchange to prepare for IEX

Combining techniques for antibody purification with regards to yield and purity. Steps in circles are optional and may only be applied on an as required basis. AC = affinity chromatography, CIEX = cation ion exchange chromatography, AIEX = anion exchange chromatography, SEC = size exclusion chromatography.

Featured products

MabSelect™ PrismA

These HiTrap™ columns are prepacked with MabSelect PrismA protein A chromatography resin. This affinity resin in the packed column has been improved with an optimized high-flow agarose base matrix and a genetically engineered protein ligand, allowing efficient cleaning between monoclonal antibody purification runs. This allows future demands in monoclonal antibody purification to be met, including processing of many bispecific antibodies.

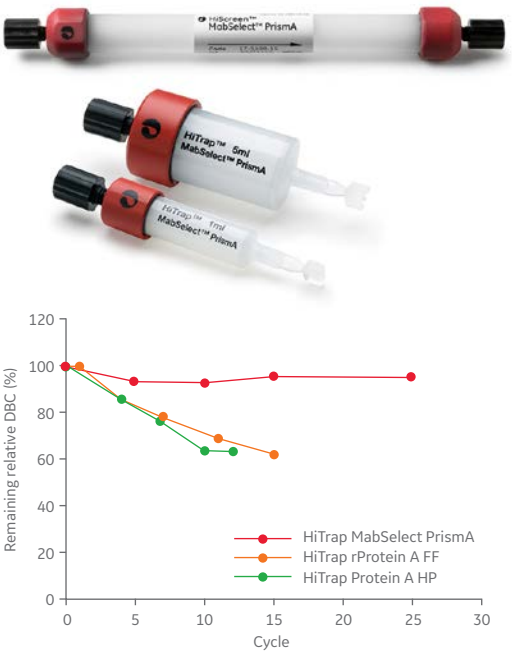
- Enhanced dynamic binding capacity compared with other protein A resins
- Excellent alkaline stability enables efficient cleaning and sanitization using 0.5 to 1.0 M NaOH

Convenient HiTrap format for easy connection to a syringe, peristaltic pump or chromatography systems such as an ÄKTA system for convenient process optimization.

Free wall poster!

So how do you best combine chromatography techniques to obtain the right purity and yield of your protein? Whether you want to purify a tagged, antibody or native protein our free wall poster helps you effectively combine the main chromatography techniques to obtain a powerful purification protocol.

*Request your poster **here!***



DBC of MabSelect PrismA, Protein A Sepharose™ High Performance, and rProtein A Sepharose Fast Flow for a polyclonal human OgG after multiple cycles with 1 M NaOH included in each cycle.

Try our new Benchkote™ sheets for ÄKTA avant, pure or start and protect top buffer tray from buffer spillages and salt deposits.

Click **here** for more information.

Ordering information

Resin	Format	Description	Volume	Pack size	Item	Price* (€)
–	–	ÄKTA start system	–	1	29022094	6 627,00
–	–	Frac 30 collector	–	1	29023051	1 299,00
–	–	UNICORN™ start 1.1 (DVD + activation code)	–	1 DVD	29276964	1 121,00
MabSelect PrismA	Pre-packed columns	HiTrap MabSelect PrismA	5 mL/column	5 columns	17549854	4 212,00
MabSelect PrismA	Pre-packed columns	HiTrap MabSelect PrismA	1 mL/column	5 columns	17549852	1 072,00
Sephacryl™ S-200 HR	Pre-packed columns	HiPrep™ 16/60 Sephacryl S-200 HR	120 mL	1 column	17116601	639,00
Sephacryl S-300 HR	Pre-packed columns	HiPrep 16/60 Sephacryl S-300 HR	120 mL	1 column	17116701	639,00
Superdex™ 200 Increase	Pre-packed columns	Superdex 200 Increase 10/300 GL	24 mL/column	1 column	28990944	2 140,00
Sephadex	Pre-packed columns	HiTrap desalting column	5 mL/column	5 columns	17140801	229,00
RC membrane	Non sterile	Protein Prep syringe filter for ÄKTA systems	13 mm 0.45 µm	150/pk	10463113	332,00
RC membrane	Non sterile	Protein Prep syringe filter for ÄKTA systems	13 mm 0.2 µm	150/pk	10463103	332,00
RC membrane	Non sterile	Protein Prep syringe filter for ÄKTA systems	30 mm 0.45 µm	150/pk	10463033	365,00
RC membrane	Non sterile	Protein Prep syringe filter for ÄKTA systems	30 mm 0.2 µm	150/pk	10463043	365,00

*For more information please 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

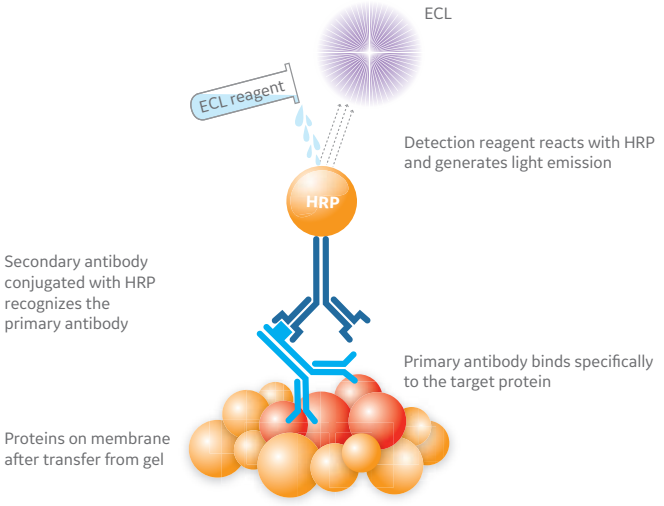
In most contemporary ECL™ systems a luminol peroxide detection reagent is added to the membrane and reacts with the horseradish peroxidase enzyme (HRP) conjugated to the secondary antibody. HRP catalyzes the oxidation of luminol in a multistep reaction and is accompanied by the emission of low-intensity light at 428 nm, which can be measured with light-sensitive X-ray film or with a CCD imager.

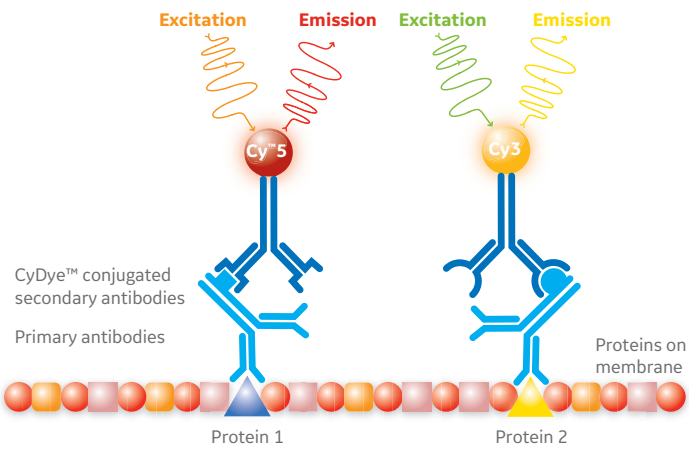
Fluorescence

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.





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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

GE Healthcare Life Sciences offers 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	Price* (€)
Chemiluminescent	Reagent	ECL Western blotting detection reagent	For 2000 cm² membrane	1 pack	RPN2209	241,74
Chemiluminescent	Reagent	ECL Select WB detection reagent	For 1000 cm² membrane	1 pack	RPN2235	312,12
Chemiluminescent	Reagent	ECL Prime Western blotting detection reagent	For 3000 cm² membrane	1 pack	RPN2236	527,34
Chemiluminescent	Kit	Amersham QuickStain kit	1 µg/mL to 20 mg/mL	1 pack	RPN4000	122,13
Chemiluminescent	Kit	Full range Rainbow molecular weight marker	250 µL	1 pack	RPN800E	221,34
Chemiluminescent	Sheets	Amersham Hyperfilm ECL	5 × 7 inches	1 pack	28906835	183,00
Chemiluminescent	Roll	Amersham Hybond™ PVDF	0.2 µm 260 mm × 4 m	1 roll	10600021	341,33
Chemiluminescent	Roll	Amersham Protran™ NC	300 mm × 4m 0.45 µm	1 roll	10600016	323,34
–	Sheets	GB003 blotting paper	460 × 570 mm	50/pk	10427826	514,55

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

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* GE recommended list prices.

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