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GE Healthcare

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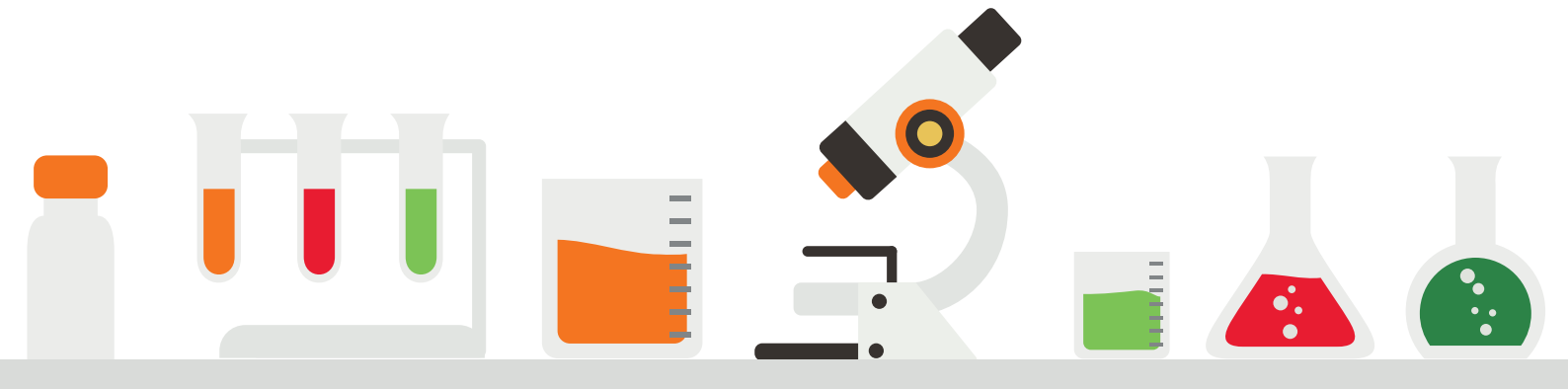

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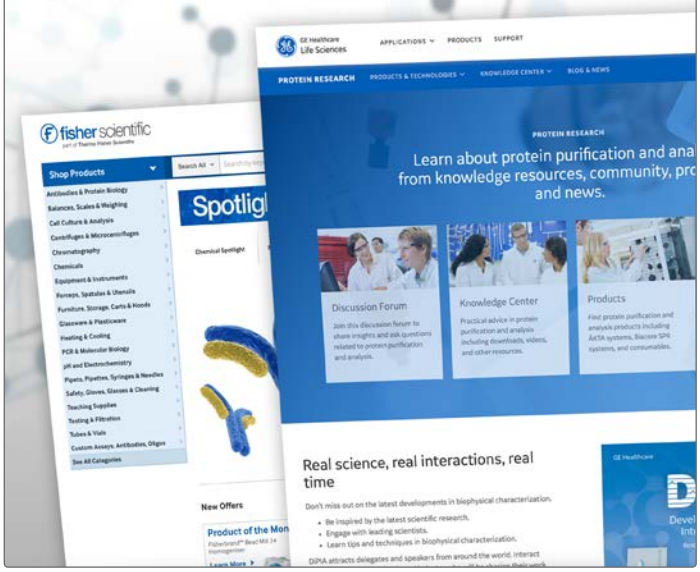

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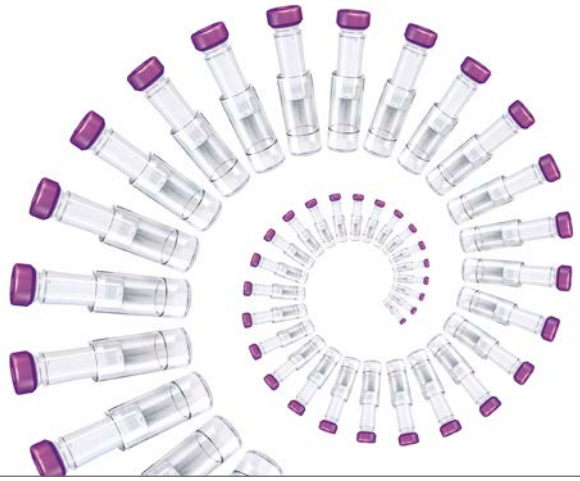

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What is single-cell sequencing: challenges and applications

Drilling down to the single-cell level provides insights into complex biological systems and diseases. Learn about single-cell sequencing, its applications and challenges. By GE Healthcare.

Single-cell sequencing: an overview

Standard bulk methods of cell analysis use many thousands of cells. When we analyze that data, we're effectively averaging out any small cell-to-cell variances and concentrating on features or data points that rise above the noise.

It's easy to assume that all cells of the same timepoint from the same sample are, well, the same. But that's far from the case. Cell populations are heterogeneous: studying single-cell samples is crucial to understanding these complex biological systems. Drilling down to the single-cell level allows us to understand the effects on and contributions by individual cells within their environment.

What is single-cell genomics?

Single-cell genomics applies standard analytical techniques, including sequencing and microarrays, to the individual cell, utilizing advanced techniques for selecting and handling individual cells and maximizing the raw material (DNA, RNA, proteins) held within. Single-cell genomics has numerous applications in both basic research and clinical settings.

Single-cell genomics: applications

- The heterogeneity of solid tumors is well-known. Single-cell sequencing gives researchers the ability to study individual cells from various points in a tumor's progression and its micro-environment, and opens up investigative pathways that may lead to better diagnostics, treatments and cures.
- In cases where a direct biopsy would be invasive, single-cell sequencing enables clinicians to detect and monitor circulating tumor cells (CTCs), which present cancer biomarkers that can direct treatment, minimizing therapies that are unlikely to succeed.
- Liquid biopsies, such as in non-invasive pre-natal testing (NIPT), are also quite well-established for sequencing cell-free DNA. In this example of liquid biopsy, NIPT can detect various genetic conditions from fetal DNA that circulates in the mother's bloodstream, avoiding invasive testing.
- Single-cell genomics can be an essential tool in forensic applications where a few cells might be all you have to work with from a casework sample.

As the technology improves and becomes more accessible, the areas of application will only expand. Single-cell genomics is already allowing archaeologists, anthropologists and paleontologists to utilize genomics in new and interesting ways.

Single-cell genomics: challenges

Three core processes in single-cell sequencing present challenges which can also affect sequencing outcomes.

- Selecting and handling individual cells
- Extracting DNA (and/or cDNA) from the cells
- Amplifying the genetic material.

Careful **handling** through manual pipetting might be the way to go if you're working with a few individual cells. However, If you need to analyze a large sample at the single-cell level, this approach is quite labor intensive. In that case, microfluidics might be the best option. Recent developments enable these systems to handle thousands of cells in parallel.

Current **extraction** techniques are quite robust, though they do need careful control for efficient release and high yield of material for amplification.

Amplification is the most challenging process in single-cell sequencing. While there are several methods available, each technique can introduce bias that may affect your results.

Single-cell sequencing: methods of amplifying DNA

There are currently three general approaches to amplifying DNA:

- PCR*-based amplification (Polymerase chain reaction-based amplification)
- Multiple displacement amplification (MDA)
- Combinations of PCR and MDA, such as MALBAC

All these methods have pros and cons, but it's possible to manage the limitations with an understanding of how they work, and where the limitations come from.

PCR uses thermal cycling to induce DNA replication. Unfortunately, it is prone to variations in reliability across different loci, and false positives and negatives in analysis. This method has fallen out of use.

MDA amplifies DNA through multiple displacement, binding primers to newly-formed DNA while polymerization is still ongoing. MDA with generic primers and DNA polymerase Phi29 can amplify picograms of DNA to micrograms, more than enough for NGS. Phi29's high fidelity results in low rates of false positives and negatives in analysis, making it well-suited for identifying single nucleotide polymorphisms (SNPs) and other mutations. MDA is the most popular current method of amplification, but it can create non-uniform representation of genomic regions.

The newest approaches to amplifying DNA, such as MALBEC, aim to use elements of both PCR and MDA, while mitigating the drawbacks of both methods. Although these hybrid methods do improve uniformity, they also depend on PCR, and have some of the drawbacks that entails, including a higher rate of false positives and negatives.

Whichever method you choose, once you have your starting material amplified, generating a library, sequencing, and analysis are relatively straightforward, and not too different from bulk cell analysis.

Single-cell sequencing experts can help!

If you'd like support with your single-cell genomics application, get in touch with GE scientific support. Or learn more about GE's solution for Phi29-based DNA amplification, GenomiPhi, which can solve common challenges when performing single-cell sequencing.

*Click **here** to view the full article.*

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

Featured products

illustra™ single cell GenomiPhi™ DNA amplification kit for WGA from a single cell

When you are analyzing the genome of a single cell, you are pushing the boundaries to gain unprecedented biological insights. You need a whole genome amplification (WGA) method that delivers results you can trust.

- Phi29 DNA polymerase for isothermal multiple strand displacement amplification (MDA).
- Highly uniform amplification over the entire genome.
- Clean manufacturing process ensures components are free of detectable DNA contamination.
- Proprietary enzymatic clean-up step removes DNA contamination introduced before amplification.
- Optimized lysis protocol promotes complete cell lysis with uniform DNA release.
- Greater accuracy than existing PCR based WGA methods.
- The amplified DNA is suitable for common downstream applications, including PCR, SNP array genotyping, array CGH, and next-generation sequencing.

Genomics resources for your lab

We're committed to helping you get the most from your molecular biology workflows. Explore gene function, regulation, and variation with our broad range of kits, reagents, and educational resources focused to your needs.

Check GE knowledge centre.

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Production of recombinant monoclonal antibody (*r*mAb) in Chinese hamster ovary cells using HyClone CDM4CHO medium and HyClone Cell Boost™ 2 feed supplement

One of the most important cell lines used in the production of recombinant proteins is the Chinese hamster ovary (CHO) cell line. Regulatory concerns surrounding the use of animal-derived components in the production of therapeutic proteins is a major driver for the development of chemically defined and animal-derived component-free (ADCF) media for CHO cell growth and protein production. This application note demonstrates the performance of the chemically defined HyClone CDM4CHO base medium optimized for CHO cells. To increase process yield, the CHO cell culture was supplemented with HyClone Cell Boost 2.

Introduction

HyClone CDM4CHO is a CHO cell culture medium free of protein and animal-derived components. This regulatory-friendly medium is developed to increase process yields for the industrial manufacture of recombinant proteins using a variety of CHO cell clones. This medium has been successfully tested in a variety of culture systems, including T-flasks, shaker flasks, and bioreactors including fed-batch and perfusion culturing. In this study, an *r*mAb-producing CHO cell clone was cultured in a stirred-tank bioreactor.

To optimize process yields with HyClone CDM4CHO medium, the culture was fed HyClone Cell Boost 2 supplement. This supplement is designed to provide nutrients such as carbohydrates, amino acids, and vitamins as part of a fedbatch culture strategy, and has been developed for recombinant protein production with various cell lines including CHO cells.

Materials and methods

An *r*mAb-producing CHO cell line was used in this study. Cells were grown in 1.5 L suspension cultures in HyClone CDM4CHO medium using a 3 L stirred-tank bioreactor (Applicon). Culture temperature was controlled at 37°C, dissolved oxygen was controlled at 50% air saturation, and culture pH at 7.0.

The culture was pulse-fed at 40 mL/L with HyClone Cell Boost 2 supplement (hydrated at 149 g/L in injection-grade water and pH adjusted to ≥ 9.5) on day 4 to 8.

Results

Viable cell density reached a maximum of 6.9 × 10 cells/mL within the culture span of 13.5 days (Fig 1). The *r*mAb yield for the process was 0.86 g/L. Glucose was added to the culture as needed when fed with HyClone Cell Boost 2 supplement. Glucose and lactate profiles are shown (Fig 2). As shown, cells started to metabolize lactic acid after day 5. Using the described fedbatch process, productivity was improved 2–4-fold compared with initial production levels (0.1796 g/L) in batch mode (Fig 3).

Conclusion

CHO cells were successfully grown in fed-batch suspension culture utilizing HyClone CDM4CHO medium supplemented with HyClone Cell Boost 2. Compared with initial production levels in batch mode, a significant productivity improvement could be achieved with the described fed-batch process.

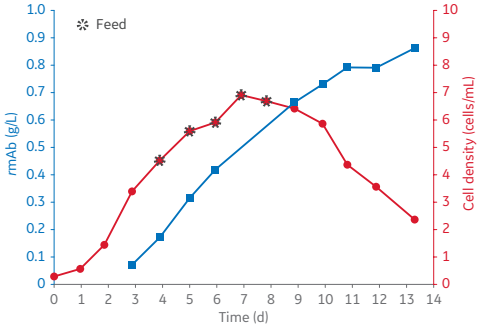


Fig 1. Cell density and *r*mAb production in fed-batch CHO cell culture using HyClone CDM4CHO base medium and HyClone Cell Boost 2 feed supplement.

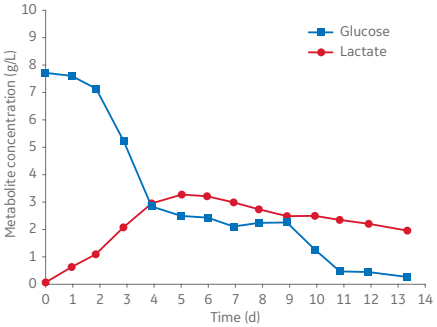


Fig 2. Glucose and lactate profiles of CHO cells in fed-batch cultures using HyClone CDM4CHO base medium and HyClone Cell Boost 2 feed supplement.

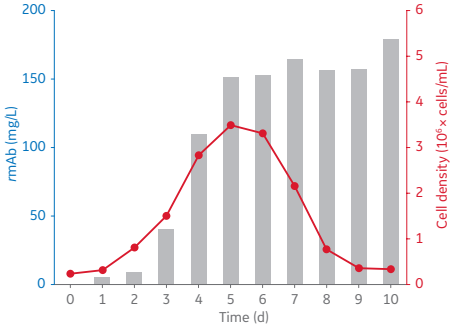


Fig 3. Cell density and *r*mAb production in batch CHO cell culture using HyClone CDM4CHO base medium.

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

Featured products

HyClone Fetal Bovine Serum FBS from origins USA, Australia and New Zealand

HyClone Defined Fetal Bovine Serum (FBS) is GE Healthcare's highest quality FBS and is widely used by cell culturists who have a concern for viral contaminants and require an extensive biochemical profile.

HyClone defined FBS is filtered through serial 40 nm (0.04 µm) pore size-rated filters, which are the most retentive filters used in commercial FBS production. This type of filtration is a practical, cost-competitive method of viral load reduction. Data shows that 40 nm filtration will remove as many as eight logs of viral challenge. Studies demonstrate that this filtration regimen has no adverse effect on cell growth. Before dispensing, each lot of serum is pooled using true pool technology to ensure uniformity and consistency between bottles. HyClone Defined FBS has over 50 components analyzed on the finished product and the results are included in the certificate of analysis and the biochemical assay list.

HyClone Cell Boost supplements

HyClone Cell Boost supplements each provide an exclusive selection of nutrients such as amino acids, vitamins, lipids, cholesterol, glucose and/or growth factors in complements optimized for multiple mammalian cell types.

All HyClone Cell Boost supplements are chemically defined and contain no animal derived components. The HyClone Cell Boost supplements are designed to increase cell productivity in a variety of cell lines.

HyClone Cell Boost Supplements	Cell type	Amino acids	Vitamins	Glucose	Trace elements	Growth factors (peptides)	Hypoxanthine/ thymidine	ADCF lipids	ADCF cholesterol	Catalog #
Cell Boost 1	CHO, HEK293	•	•	•						SH30584
Cell Boost 2	CHO, PER.C6	•		•						SH30596
Cell Boost 3	Hybridoma, myeloma	•	•	•	•		•			SH30825
Cell Boost 4	CHO	•	•	•	•	•		•	•	SH30857
Cell Boost 5	CHO, HEK293, Hybridoma, NS0	•	•	•	•	•	•	•	•	SH30865
Cell Boost 6	CHO, HEK293, Hybridoma, NS0, T-cells	•	•	•	•	•	•	•	•	SH30866

HyClone WFI quality water to either US or EU specifications

Water for injection (WFI) quality water is widely used in the pharmaceutical industry. Today's rapid advances in biotechnology and biopharmaceutical manufacturing create an ever-increasing need for dependable sources of WFI quality water. Whether your manufacturing need is large volumes of WFI quality water for cell culture processes, for cleaning and rinsing, or for buffer preparation, we have the resources, expertise, and capacity to be your partner of choice.

HyClone HyPure WFI Quality Water:

- Meets stringent Pharmacopeial specifications.
- Manufactured in an ISO 9001 certified facility.
- Certificate of analysis (CoA) for each lot of water produced.
- Low levels of endotoxin.
- Sterile filtered.

For comprehensive upstream cell culture and bioprocessing toolbox for pharmaceutical research, visit ***GE Knowledge centre.***

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

Automation in dissolution testing

Dissolution testing is an essential process in the pharmaceutical industry for evaluating the rate of release of an active pharmaceutical ingredient (API) from its dosage form. Automated solutions for this process allow laboratories to benefit from improved accuracy, precision, and productivity. Filtration plays a key role in sampling for dissolution testing. The selection and use of filters in the workflow can affect data consistency and accuracy

Why automate dissolution testing?

Dissolution testing can be performed manually or using semi- and fully-automated systems. In all cases, it is necessary to comply with specifications outlined in the relevant pharmacopeia.

Automating all or part of the workflow minimizes variables that might otherwise contribute to out-of-specification results. Automation might be involved in:

- Preparation and dispensing of dissolution media
- Introduction of the dosage form
- Sampling
- Preparation of the sample for analysis
- Data collection and analysis

Filtration in sampling

Autosamplers can help maximize data consistency in dissolution testing. As the name suggests, autosamplers automatically draw samples from the dissolution vessel at specified time points. These instruments then prepare samples for injection into analytical instruments, often high-pressure liquid chromatography (HPLC) systems.

Filtration of the sample, either through cannula filters at the tip of sample probes or via in-line syringe-type filters, halts the dissolution process and separates any undissolved API and excipients.

The US Pharmacopeia (USP) General Chapter 11 (2011) specifies that sampling and filtration must occur within ± 2% of the stated time point. For example, a 10 min timepoint requires pulling and filtering a sample within 12 seconds of the intended time, something that is only reliably achievable through automation.

Filtration enables samples to be stored for analysis, or immediately injected into the analytical instrument without introducing particulates that could block the column frit.

Selection and use of filters

Several factors contribute to the effectiveness of sample filtration and subsequent accuracy and consistency of downstream analyses. Whether using cannula or syringe-type, a filter can vary in its suitability for a given drug formulation and dosage form.

Adsorption

The filter material can adsorb limited quantities of drug onto its surface. The rate and level of this adsorption varies between filter materials. For example, the article “Comparison of the Adsorption of Several Drugs to Typical Filter Materials” identified that regenerated cellulose (RC) and glass fiber (GF) have reduced adsorption compared with nylon.

According to a November, 2013 webinar, adsorption can artificially lower the apparent concentration of a drug in downstream analysis. Initial flushing with the sample (e.g., 2 mL) might be necessary to saturate the filter before taking a representative sample.

If a filter device is used for more than one time point for the same sample, flushing will not be necessary at every time point. Validation tests can identify the sample volume that needs to be flushed and the number of samples that can be filtered before clogging.

These tests enable analysts to set up an autosampler to flush and replace filters automatically according to the standard operating procedure (SOP).

Pore size/efficiency

The efficiency of a filter will vary depending on the size of undissolved drug and excipient particles.

Validating pore size for each drug dosage form during method development can help to maximize filtration efficiency and reduce particulates entering downstream analysis instruments.

Agitating samples encourages any particulates to dissolve. Drug levels compared between agitated and unagitated samples after filtration can be used to validate the efficiency of filtration.

Chemical compatibility

Dissolution media varies depending on the simulated environment required. The combination of pH, ionic strength, surfactant, and other factors can influence the choice of filter material.

Selecting filter materials with broad chemical compatibility, and validating against various media, will simplify selection of filtration devices across different sample types.

Materials better suited to aqueous samples, for example, will resist non-aqueous media. The resulting back pressure can slow manual and automated filtration, and affect data accuracy. Forcing a sample through an incompatible filter might, in rare cases, risk damaging the membrane.

Polytetrafluoroethylene (PTFE) and RC are compatible with a range of solvents. Specifically, PTFE is a hydrophobic material compatible with organic solvents, strong acids, and alkalis. RC on the other hand is a hydrophilic material, compatible with aqueous solutions and organic solvents.

Extractables

Any filter material, or indeed any component of a system, has the potential to release extractables when it encounters dissolution media. Extractables only become an issue if they absorb at the same wavelength as the API.

Comparing the UV spectra of filtered and unfiltered samples can reveal any influence of extractables on drug measurement.

RC is an example of a material that generally has low levels of extractables, making it well suited for preparing various sample types prior to HPLC.

Excipient load

As stated in a November, 2013 webinar some drug dosage formulations produce high levels of excipients during dissolution testing. These excipient particles can quickly clog filters, affecting filtration efficiency and the accuracy of analyses.

Where clogging is a risk due to high excipient load, stacked filters containing GF pre-filters can help. Designed for difficult-to-filter samples, the GF traps coarse particles, preventing them from reaching and clogging the final membrane.

Filter selection influences the accuracy and reliability of dissolution data. An initial validation identifies the various influencing factors, helping to select the most appropriate filter based on the properties of the API and dissolution media.

Try our Whatman™ Filter Selector App to find out if you are using the most appropriate filtration solution for your samples. To discuss any challenges you are facing, please contact GE's Life Sciences Scientific Support.

*Click **here** to view the full article.*

Featured products

Roby automated syringe filters

Roby syringe filters are designed specifically for automated filtration systems. The design of the polypropylene housing ensures smooth transport from the storage turntable to the filtration site.

The Roby filter validation kit offers six different Roby automated syringe filters, along with a filter validation protocol and filter selection aid. Tubes of 25 filters are ready for loading into tablet tester systems.

850-DS 8-channel filter plate

Whatman filter plates for use in Agilent™ 850-DS Dissolution Sampling Station.

Automated processing of up to 8 samples simultaneously. The filter plates are specially designed for Agilent equipment and increase productivity by allowing reliable alignment of the liquid path and reducing the risk of jamming or leaks that may occur with other dissolution sample preparation systems.

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

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

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

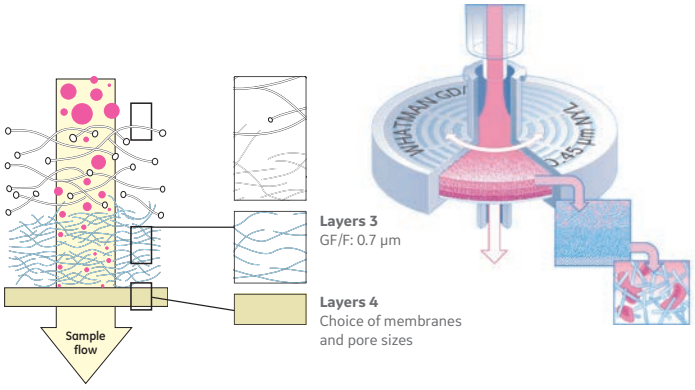
When your lab prepares a wide variety of sample types using different solvents for HPLC analysis, identifying appropriate membrane materials can be time-consuming. Different materials might be more or less suitable for a given sample based on chemical compatibility and solvent resistance.

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.

Try our Whatman Filter Selector App to find out if you are using the most appropriate filtration solution for your samples. To discuss any challenges you are facing, please contact GE's Life Sciences Scientific Support team.



Whatman GD/X™ stacked syringe filter

Mini-UniPrep™ syringeless filters

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

Guide to laboratory filtration

Filtration devices for small volume sample preparation

Select the optimal Whatman filter for your application

Step 1: Choose application
Step 2: Choose appropriate filter

Puradisc FP
3* 4 9*
11 14
*Notes:
3 and 9: CA

ReZist™
1 4 7 14

Puradisc Aqua 30
12 13

Start here

Protein Prep for ÄKTA™
9

Puradisc
3* 4 7 9*
11 12* 13* 14
*Notes:
3 & 9: CA, PES, PVDF
12 & 13: PES

Anotop™
3 4 6 7 8
9 10* 11 14
*Notes: 0.02 µm

Mini-UniPrep G2
2 7

Anotop Plus
4 5 7 10*
*Notes: 0.02 µm

Mini-UniPrep
2 7

Roby
2

GD/XP
4 5 7 8
12 13 14

Uniflo™
3 4 7 11
12 13 14

SPARTAN™
4 7 9 14

Whatman GD/X
4 5
7 11 14

Applications

1. Air venting
2. Automated filtration of samples/ Tablet dissolution testing
3. Biological sample preparation
4. Capillary electrophoresis
5. Difficult to filter samples (high solid content samples)
6. Filtration of colloidal material
7. HPLC/UHPLC sample preparation
8. Ion-chromatography
9. Filtration of protein containing samples
10. Filtration of nano particles
11. Sterile filtration (use sterile filter and membrane with pore size 0.2 µm)
12. COD/TOC/DOC
13. Trace metal analysis (ICP/AAS/ICP-MS)
14. UV/VIS analysis

COD = Chemical oxygen demand;
TOC = Total organic carbon;
DOC = Dissolved organic carbon
Note: For guidance. Only a selection of applications shown above

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

Making mAbs purification efficient and cost-effective

Monoclonal antibodies (mAbs) are the largest and fastest growing class of biological drugs today. As a result, there is high demand for solutions that will deliver efficient, flexible, and cost-effective purification.

Downstream mAb purification platforms commonly include a protein A-based capture step followed by one or two polishing steps to remove remaining impurities. This offers high purity and a high degree of recovery in a single capture step.

Capture using protein A ligands

MabSelect™ is our protein A affinity family of resins designed for capturing mAbs from large sample volumes. The resins are based on a highly cross-linked agarose matrix with a recombinant protein A ligand. The figure below shows available MabSelect resins and contains suggestions for best use.

Optimizing cleaning and sanitization for improved process efficiency

Efficient cleaning prevents impurities from building up on the chromatography column and reducing the capacity of the resin. Efficient cleaning and sanitization protocols also help prevent growth of microorganisms and inactivate potential endotoxins. A high alkaline stability of the resin enables the use of high concentrations of low-cost sodium hydroxide as a cleaning agent. MabSelect PrismA exhibits more than 95% retained dynamic binding capacity after cleaning with 0.5 M NaOH between runs for 150 cycles (see figure) and still 90% with 1.0 M NaOH.

Polishing mAbs in a two-step process

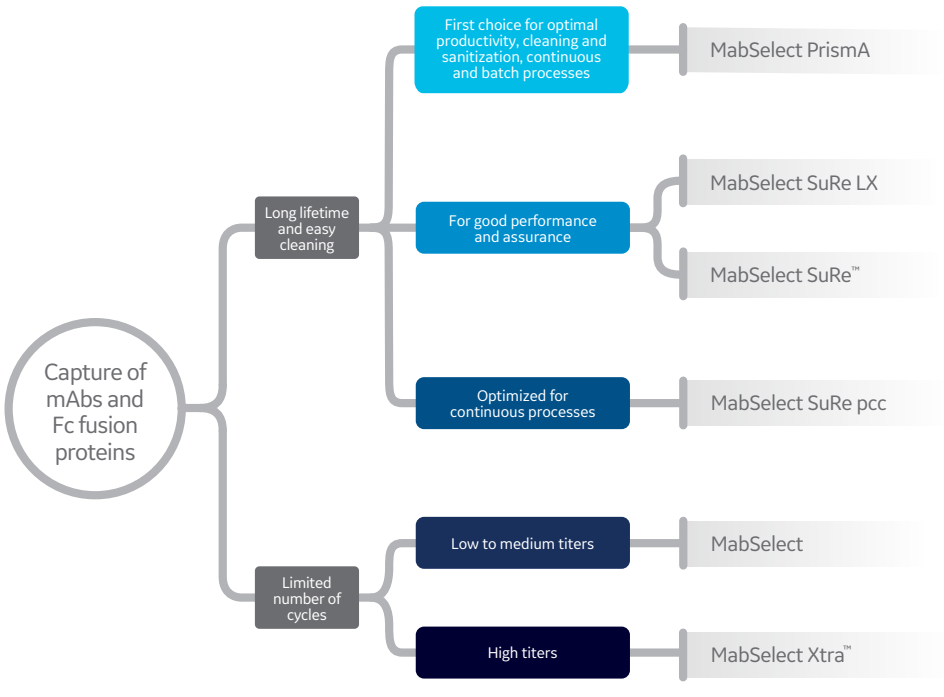
The polishing steps following the capture step can be performed in either bind-elute (binding) or flow-through (nonbinding) mode. Our Capto™ adhere and Capto adhere ImpRes resins can both be used for polishing in a two-step process in flow-through mode. They are both highly efficient in removing remaining impurities like aggregates, host cell protein (HCP), DNA, and viruses.

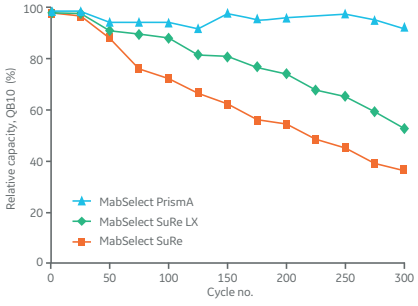
Polishing mAbs in a three-step process

A three-step purification process, with two polishing steps based on one cation exchanger and one anion exchanger, is a classical way of purifying mAbs.

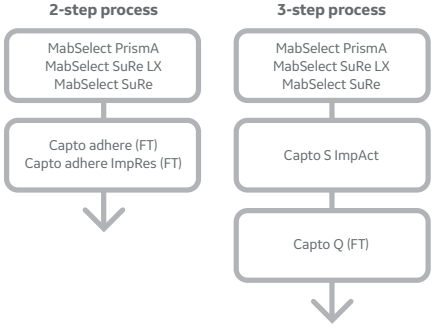
Cation exchangers are used for the removal of HCP, protein A, aggregates, and fragments. The cation exchange step is commonly followed by an anion exchange step (run in flow-through mode) for removal of remaining impurities such as DNA.

Chromatography resins and membranes suitable for polishing in a three-step purification process are Capto S ImpAct and Capto Q.





Relative remaining capacity (Q_{B10}) for 300 cycles, including CIP with 0.5 M NaOH for 15 min/cycle.



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Featured products

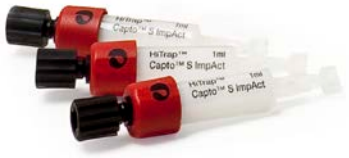
MabSelect PrismA

- Enhanced dynamic binding capacity allows high mass throughput of processed mAb per resin volume unit.
- Excellent alkaline stability enables efficient cleaning and sanitization using 0.5–1.0 M NaOH for improved process economy, bioburden control, and robustness.

Capto adhere and Capto adhere ImpRes

- Wide operational window of pH and conductivity.
- Two-step chromatographic process with protein A affinity, saves time and cuts operating costs.

Capto S ImpAct



- High binding capacity, > 100 mg mAb/mL resin.
- Efficient aggregate removal at high load of monoclonal antibodies.
- High-resolution polishing based on the well-established Capto platform.

Capto Q

- High dynamic binding capacity at high flow raises productivity.
- High-volume throughput cuts process times.
- Cost-effective processing with smaller unit operations.

Sample preparation with the Protein Prep syringe filter for ÄKTA™ systems

NEW

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.

*Request your sample **here**.*

Protein Prep syringe filter for ÄKTA systems

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

DIBE technology for reliable HCP detection

Host cell protein (HCP) is a primary impurity and a critical quality attribute (CQA) for biopharmaceuticals (biologics). HCP affects product quality, safety and efficacy. HCP ELISA is the gold standard of HCP detection and measurement, which requires polyclonal Antibodies (Ab) with broad reactivity against a wide range of potential HCPs.

Regulatory authorities require the characterization of the ELISA Abs used in the HCP ELISA assay. 2-D gel electrophoresis followed by Western blotting is the recommended approach to characterize HCP ELISA antibodies and their coverages.

2D differential in blot electrophoresis (2D DIBE) combined with Western blotting is a powerful technology for separation and visualization of complex protein mixtures such as HCPs.

High sensitivity—Fluorescent multiplexed methodology based on CyDye™ pre-labeled Western blotting, and image acquisition with Amersham™ Typhoon™ laser scanner deliver high sensitivity for HCP detection.

Minimal variation—Labeled proteins can be directly compared to the proteins detected by CyDye pre-labeled antibodies on the same membrane.

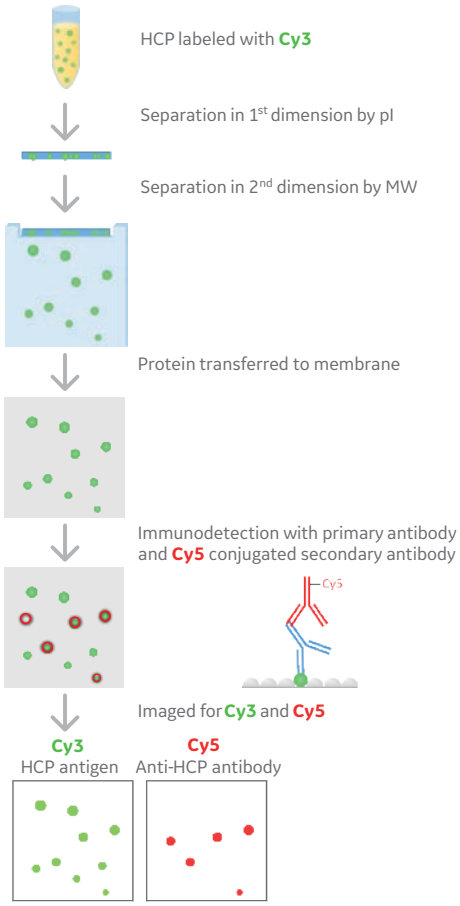
No mismatches—Multiplex fluorescence image acquisition with the Amersham Typhoon simultaneously captures both HCP antigen and anti-HCP antibody images from a single membrane.

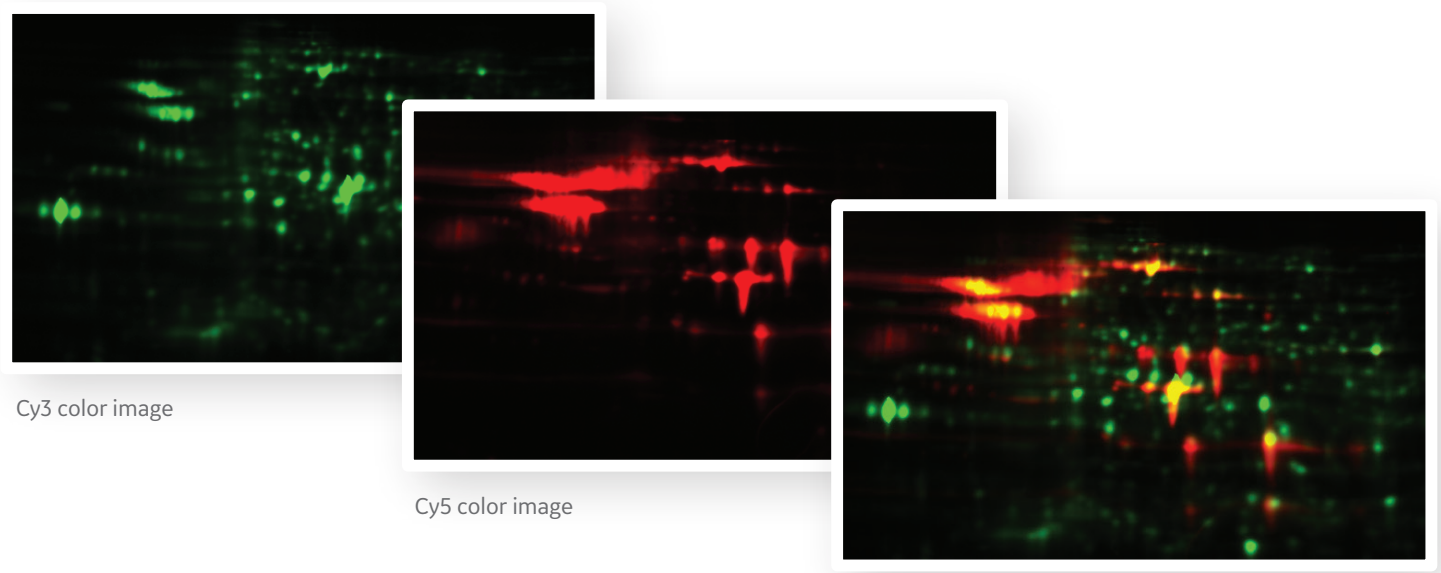
Fast evaluation—Melanie™ Coverage software helps investigators evaluate the data in less time, with greater confidence.

2D DIBE for HCP coverage assay

Total HCPs are labeled with CyDye DIGE Cy™3 minimal dye. After 2D electrophoresis, protein spots are transferred onto a PVDF membrane. The HCP antibody is applied to the membrane and visualized by Western blot with Cy5 fluorescence. Those two images are then overlaid. Cy3 labeled total HCP spot and Cy5 immunodetected spot overlay is confirmed by Melanie Coverage analysis software with 3D visualization. Finally, Melanie Coverage software provides a coverage percentage value for this assay.

With the goal of helping you achieve the best results, we deliver 2D DIBE products that improve data quality when compared to traditional 2D experiments and Western blotting, and can be integrated into a complete HCP analysis solution.





Cy3 color image

Cy5 color image

Cy3 and Cy5 color overlay

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

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%.

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.

For complete list of products to support your western blotting applications click **here.**

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

Distributor
GE Healthcare

All offers in this issue are valid until 31 December 2019.

* The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and F Hoffmann-La Roche Ltd. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers such as GE Healthcare and affiliates when used in conjunction with an authorized thermal cycler. Phi 29 DNA polymerase and its use for DNA synthesis is covered by US patent numbers 5,854,033, and 5,576,204. Ready to Go RT-PCR Beads: Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, and 6,127,155. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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