The Selection of Depth Filtration Devices for Cell Cultures, Fermentation Broths and Cell Lysates

Introduction

Solutions derived from cell cultures, fermentation broths and cell lysates often contain large amounts of particulate load. The clarification of cell culture solutions is required to remove particles prior to further downstream processing such as ultrafiltration or capture chromatography.

Particulates, such as cell debris, may vary in size and can be difficult to filter using a standard membrane filtration device. Membrane filters are typically thin, highly porous materials that have a narrow pore size distribution, this prevents particles larger than the rated pore size from passing through the filter. During filtration particulate matter larger than the pore size will build up on the membrane surface, eventually clogging the open pores of the structure until no more solution can be filtered. Therefore, when filtering solutions that contain large amounts of particulate, such as cell culture solutions, a standard membrane filter can quickly block and need replacing.

Devices containing depth media filters can typically offer a more efficient filtration for solutions with heavy particulate loads. Figure 1 highlights the differences between the structures of membranes and depth media materials. Depth media is thicker than filter membranes, so a range of particulate of different sizes can be trapped on both the surface and throughout the matrix of the filter media.

Figure 1

Depth Media

- Consists of either multiple layers or a single layer of a medium having depth
- Captures a range of particulate sizes that can be trapped on both the surface and throughout the matrix of the filter media

Membrane Filters

- Thin, highly porous materials that have a narrow pore size distribution, typically trap contaminants larger than the pore size on the surface of the membrane
- Membrane filters are commonly used for critical applications such as sterilizing and final filtration

Pall supplies a variety of depth filtration products and devices for the downstream processing of cell culture solutions, fermentation broths and lysates. These devices are suitable for a range of different volume throughputs, and are designed to suit different handling and application workflow requirements.

These products include filter plates which are ideal for the processing of small sample volumes in high-throughput applications. Pall also offers syringe filters and larger scalable capsule devices which are ideal for larger volumes of sample.

Volume 100 µL - 2 mL
AcroPrep™
Filter Plates

Volume < 7 mL
AcroPrep™
24-Well Filter Plates

Volume < 200 mL
Syringe Filters with
Glass Fiber Media

Volume < 100 L
Supracap™
Depth Filter Capsules

This scientific brief will summarize the important factors to consider when selecting the most optimal depth filtration device to use for laboratory workflows.

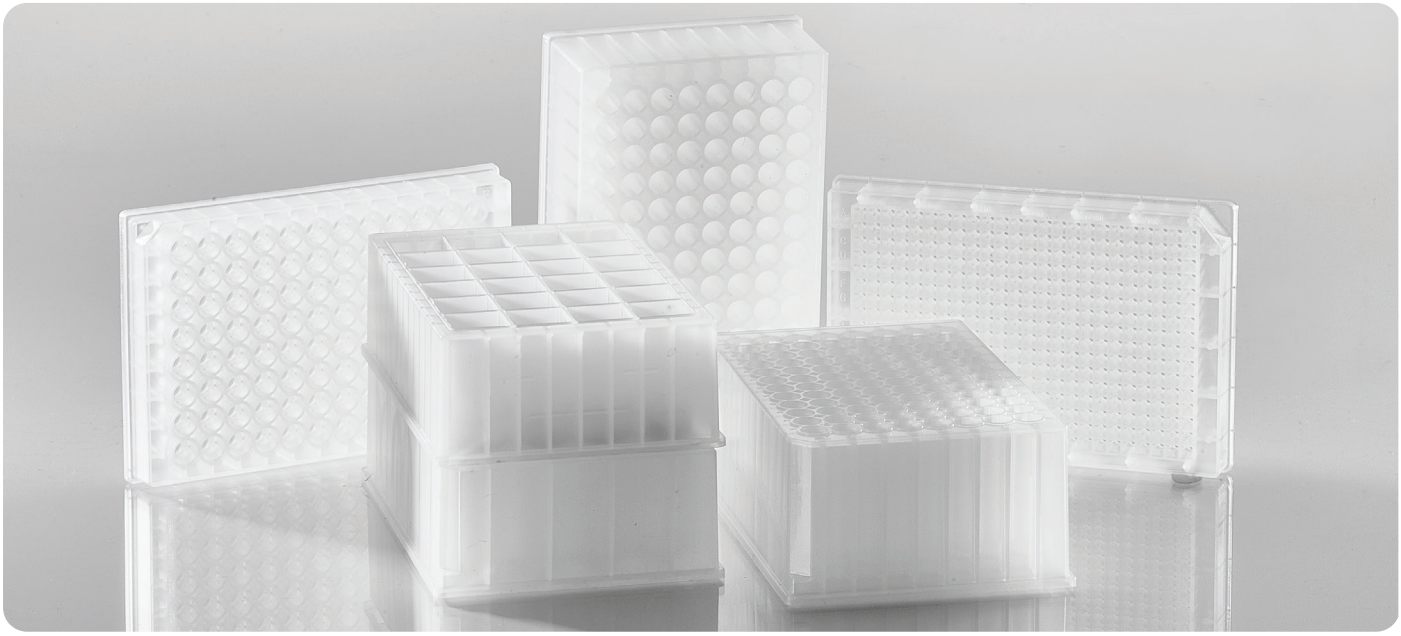
Filter Plates

As samples get smaller and more numerous multi-well plates have become a standard tool in many laboratories, allowing scientists to undertake high-throughput research, processing numerous samples all in one go, often on an automated robotic platform.

In the 1990s, Pall introduced their first multi-well filter plate range enabling scientists to perform purification steps in a high-throughput footprint. Today Pall supplies filter plates in 24, 96 and 384-well formats. These plates can easily be used on vacuum systems or with centrifugation, either manually or on automated platforms.

Table 1 - AcroPrep Filter Plates Recommended Working Volumes

	Well Volume (max)	Recommended Working Volume
AcroPrep 24-well Filter Plates	7 mL	7 mL for vacuum 6 mL for centrifugation
AcroPrep Advance 96-well Filter Plates	350 µL	≤ 300 µL
AcroPrep Advance 96-well Filter Plates, 1 mL	1 mL	≤ 900 µL
AcroPrep Advance 96-well Filter Plates, 2 mL	2 mL	≤ 1.9 mL
AcroPrep 384-well Filter Plates	100 µL	80 µL



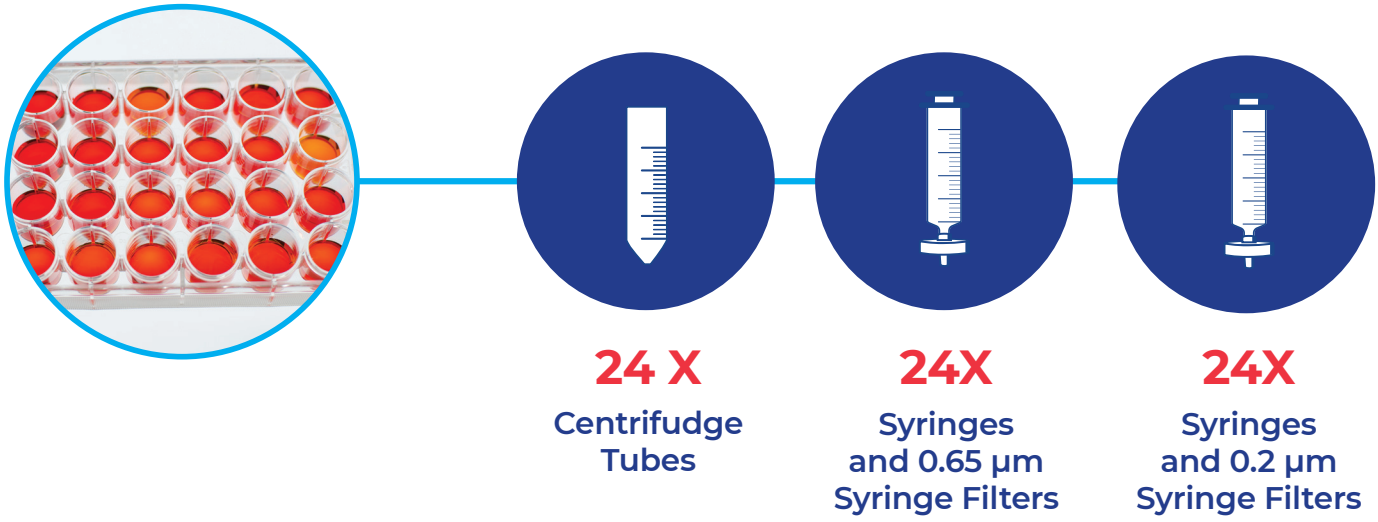
24-well Plate Format

24-well plates are a common format used in cell culture techniques. The use of 24-well culture plates in life science applications continues to grow, it is estimated that their usage will increase by between 10-15% per annum.

Until recently the medium laboratory scale through-put clarification of complex cell cultures to recover expressed proteins required numerous steps, even when performing cell culture in a 24-well plate format.

In the traditional method centrifugation is performed in individual spin columns, after which the supernatant is carefully removed, typically using a pipette and aspiration technique. The sample then requires filtration, firstly to remove any remaining particulate and secondly to sterilize the solution. Filtration is generally performed by passing the sample through syringe filters in two steps, using two separate filters (see figure 2).

Figure 2 - Traditional Process



In all, this time consuming and tedious manual workflow often requires more than 1 hour to process a single 24-well plate of samples. In addition, each step in the clarification and sterilization process may lead to sample loss due to hold-up volumes during filtration, thus potentially impacting protein recovery. Each additional step in a process can increase the potential for mistakes, lost samples, and process error.

This process also generates significant amounts of plastic waste. Processing of a single 24-well plate requires 24 centrifuge tubes, 24 pipette tips, 48 syringes and 48 syringe filters and numerous sample tubes.

Pall have introduced a cell clarification and sterile filtration AcroPrep 24-well filter plate that can both clarify and 0.2 µm sterile filter in a single device and workflow step.

Using either a vacuum manifold or centrifuge, high density cell cultures (such as CHO or HEK - see figure 3) can be quickly processed resulting in the capture of cells, cell debris and other biological aggregates in the filter media. The filtrate collected contains proteins and other sub 0.2 µm particles.

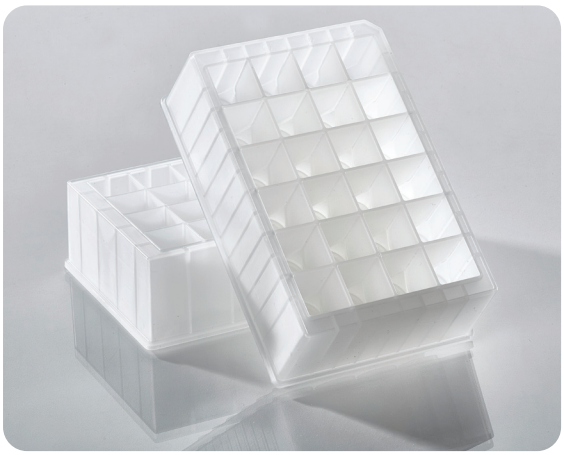
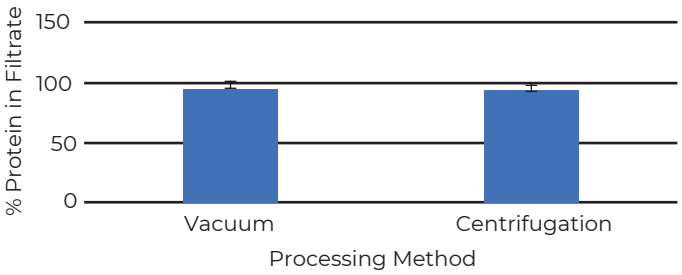


Figure 3
Protein recovery from high-density CHO cells cultures after processing through a 24-well plate (Depth + EKV media); 5 mL of CHO cells at a density of 25 M cells/mL



This innovative workflow is possible by using multi-layer filtration media and membranes integrated into one device. The top layer features Pall's Seitz® depth media and efficiently captures whole cells and cell debris. The lower Supor® EKV layer provides an efficient sterile filtration layer. Supor EKV is a hydrophilic polyethersulfone membrane that is low in protein binding and offers excellent throughputs due to its asymmetric structure.

The combination of depth filter and final filtration membrane effortlessly recovers proteins from whole cell cultures with varying viabilities of up to 25 Million + cells/mL.

Compared to traditional methods to recover proteins from cell cultures, Pall's cell clarification and sterile filtration plate uses seven times less plastic consumables by weight, significantly reducing disposal and landfill costs while also saving time and simplifying workflows. Combining the clarification and sterilization steps eliminates the need to harvest the cells in a centrifugation step, saving additional time.

The filter plates are individually bagged and gamma irradiated, both receiver plate and lid are also included for ease of use.

96-well Plate Formats

For smaller process volumes Pall supplies 96-well filter plates in 350 µL, 1 mL and 2 mL well volumes. These filter plates contain a 3.0 µm glass fiber depth filter layered on top of a Supor 0.2 µm membrane. The 3.0 µm glass fiber acts as a prefilter removing larger particulate contamination allowing for the efficient usage of the final 0.2 µm Supor membrane filter.

Presented in figure 4 is a lysate sample preparation procedure using the AcroPrep Advance filter plate for lysate clearance which effectively removes unwanted cellular debris from samples.

Filtration, which can be easily automated, is relatively quick and allows the use of additional wash steps to maximize sample recovery. Filtration can be done effectively in either vacuum or centrifugal mode, ultimately maximizing the choice in protocols available to the researcher.

Figure 4 - Protocol

For clearing lysate of *E.Coli* containing DNA plasmids

Culture growth and lysis

From Microplates

1. In each well of deep well microplate, grow 1 mL of *E.coli* containing pCAT plasmid cultures in LB + ampicillin overnight at 37 °C with shaking
2. Spin down culture plate for 10 minutes
3. Aspirate media
4. Resuspend each pellet in 100 µL resuspension buffer
5. Add 100 µL of lysis buffer to each well
6. Shake microplate for 2 minutes
7. Add 100 µL of neutralizing buffer to each well
8. Shake microplate for 2 minutes

Transfer flocculent lysate to wells of an AcroPrep Advance filter plate for lysate clearance

Proceed to clarification of lysate through vacuum or centrifugal filtration

With vacuum filtration

- Place collection plate in a Pall multi-well plate vacuum manifold
- Place filled lysate clearance plate on top of the vacuum manifold
- Apply vacuum (10 in. Hg) and start filtration
- Release vacuum

With centrifugal filtration

- Put adapter collar for centrifugation on receiver plate
- Stack filled lysate clearance plate on top of receiver plate
- Place stacked plates in a standard swinging bucket microtiter plate rotor assembly
- Centrifuge. As a general guideline, centrifugation at 1,500 x g for 1 to 2 minutes is sufficient to evacuate the well contents

Discard lysate clearance filter plate

Retrieve receiver plate

Filtrate is now ready for downstream applications

Supracap Depth Filter Capsules

Supracap depth filter capsules are available in different sizes and configurations for the processing of larger volumes of solutions.

The Supracap 50 filter capsules are the smallest volume Supracap devices and are typically used for the filtration of 1 to 3 liters. The larger Supracap 100 filter capsules come in different sizes and can be used to process up to 100 liters.

Supracap 50 filter capsules are ideal for use in developing and optimizing a process during scale-up and scale-down studies. They can be used for quickly and accurately determining which series and grade of depth media will provide the best performance as well as the necessary filtration area to meet process requirements.

Supracap 100 filter capsules are available in 5” and 10” formats. The Supracap capsules contain the same media and have the same materials of construction as Pall’s large volume, production sized devices that are capable of processing hundreds of liters of solution at a time. This allows for the easy and simplified scale-up of processes.

The capsules have the ability to be used individually or stacked in series, which can help increase throughput and decrease processing time by allowing filters of different removal ratings to work in tandem, maximizing the removal of cell debris, cell lysates and particulate matter from a solution.

Supracap Depth Filter Capsules

Choosing the Correct Supracap Depth Filtration Capsule

The optimal choice of filter media selection and capsule size is based on the type of process solution, process volume and workflow.

Firstly, we can consider filter media selection, the Supracap capsule family is available with two different media types with three distinct media configurations.

The first type of depth filtration media is Seitz BIO 20, which consists of highly purified natural and modified cellulose fibers that are free from inorganic materials. It has the tightest removal rating of Supracap filters at 0.4 to 1 µm.

The second media type found in Pall Supracap filters is the Seitz P-Series (P100 - P900), which is a more layered and complex media that is comprised of cellulose fibers, filter aids such as diatomaceous earth and perlite, and other resins that offer broader removal ranges than the BIO 20 media.

The last media type is Seitz HP-Series (PDD1 – PDP8). This media contains two layers of Seitz P-Series depth filter sheets arranged so a more permeable layer is followed by a less permeable layer to increase flow rate and flow through of high particulate solutions.

A summary of the different media types is listed below:

Bio20:

- Only cellulose
- Manufactured to have reduced levels of ash and heavy metal extractables
- Avoid release of ions into the product
- 0.4 µm final cut-off makes it useful for final filtration steps

P-Series:

- Cellulose fibers
- Filter aids (diatomaceous earth and perlite)
- Resins
- Positively charged to help removal of whole and crushed cells, cell lysate debris, endotoxins and other negatively charged host cell proteins, nucleic acids, and negatively charged viruses

HP Series:

- Seitz HP-Series: 2 layers of Seitz P-series filter media (PDD1 – PDP8)
- More permeable layer + less permeable layer
- Share the same properties as the P-series material while having a high dirt/particle holding capacity

Figure 5 and Table 2 show the pore size ranges of these media types along with specific applications that the different media types are most suited to.

Figure 5
Pore size ranges of the Pall Laboratory Supracap capsules

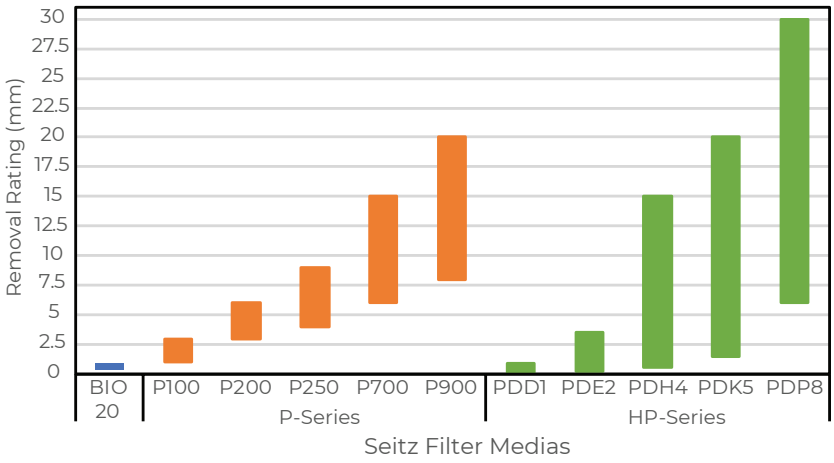


Table 2
Effective filtration area (EFA) of depth filter capsules

Device	Capsule Size	EFA (cm^2)	Connection Type	Seitz Media Type	Removal Rating (µm)	General Usage
Supracap 50	n/a	22	Luer fittings	Bio 20	0.4 µm - 1.0 µm	Final filtration, clarifying supernatant, protect columns, prefiltation to TFF
Supracap 100	5”	500	Sanitary flange or 13 mm hose barb			
	10”	1000				
Supracap 50	n/a	22	Luer fittings Sanitary flange or 13 mm hose barb	P Series (100, 200, 250, 700, 900)	1.0 µm - 3.0 µm	Cell lysate, removal of endotoxin and negatively charged bio-molecules, therapeutic protein or vaccine purification, and blood/ serum separation
Supracap 100	5”	500			3.0 µm - 6.0 µm	
					4.0 µm - 9.0 µm	
	10”	1000			6.0 µm - 15.0 µm	Retention of whole/crushed cells, general cell removal from cell culture media (bacteria, yeast, or mammalian/insect cells)
					8.0 µm - 20.0 µm	
Supracap 50	n/a	22	Luer fittings	HP Series (PDD1, PDE2, PDH4, PDK5, PDP8)	0.1 µm - 0.85 µm	Final filtration, clarifying supernatant, protect columns, prefiltation to TFF
Supracap 100	5”	250	Sanitary flange or 13 mm hose barb		0.2 µm - 3.5 µm	Cell lysate, removal of endotoxin and negatively charged bio-molecules, therapeutic protein or vaccine purification, and blood/ serum separation
					0.5 µm - 15.0 µm	
	10”	500			1.5 µm - 20.0 µm	Retention of whole/crushed cells, general cell removal from cell culture media (bacteria, yeast, or mammalian/insect cells)
					6.0 µm - 30.0 µm	

Once the correct media choice has been made we would need to consider device size. The effective filtration area (EFA) is the total area of filter media that is exposed to the process solution and usable during filtration. Typically the larger the EFA the greater the throughput of filtrate.

The Supracap 50 capsules have an EFA of 22 cm^2 and are typically used for processing 1 to 3 liters of solution. The Supracap 100 filter capsules are available in both 5” and 10” formats. The 5” capsules have an EFA of 500 cm^2 and they are typically used to filter between 3 to 50 liters of solution. The 10” capsules have an EFA of 1000 cm^2 and typically filter between 50 to 100 liters of solution. However, it should be noted that due to construction the HP series Supracap 100 capsules have half of the EFA, meaning that the 5” capsules have 250 cm^2 and the 10” capsules have 500 cm^2. EFAs are summarized in table 2.

Stacking Supracap Capsules

Maximizing total output occasionally requires stacking different Supracap devices in series. For example, Pall has designed a system for clarifying cell culture media that is well suited for monoclonal antibody (mAb) production that places a PDP8 Supracap capsule filter in series with a PDE2 Supracap capsule filter. The Seitz PDP8 HP-series filter media has a 6 – 30 µm size range (Table 2). This provides a more efficient removal of whole cells while allowing smaller debris to pass through the filter media. What flows through the PDP8 Supracap filter is then filtered by a PDE2 Supracap capsule filter. This filter combination does its job by first clarifying mAb production media with high cell counts, up to 35 million cells per mL, then removing finer particulate matter to make the solution ready for downstream processing. The use of a PDP8 Supracap filter followed by a PDE2 Supracap capsule filter is the same setup as Pall’s Stax™ mAx Clarification Platform that can handle hundreds of liter of media at a time.

This highlights the ease of scalability from small-scale runs using either Supracap 50 or 100 capsule filters to Pall’s large-scale depth filtration platforms.

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