Use of 24-Well Filter Plates for Medium Throughput High Volume Sample Preparation Applications

Georgius de Haan[1] and Nadia Kadi[2]
[1]Pall Laboratory, 20 Walkup Drive, Westborough, MA 01581, USA
[2]Pall Corporation, 5 Harbourgate Business Park, Southampton Road, Portsmouth PO6 4BQ, UK

OVERVIEW

Many techniques in Life Science research rely on efficient filtration during sample preparation. The use of filter plates has allowed simultaneous filtration of multiple samples by means of centrifugation, vacuum, or positive pressure. Until recently, this methodology was mostly available for high throughput of smaller sample volumes with the aid of 96-well or 384-well filter plates whereas processing of larger sample volumes was relegated to the use of, for instance, centrifugal devices or syringe filters. The latter devices are capable only of handling individual samples, complicating simultaneous processing of multiple samples. Pall has recently introduced a family of 24-well filter plates which allow simultaneous processing of samples with volumes of up to 7 mL, for applications as depth filtration/clarification, (sterile grade) microfiltration, and ultrafiltration, thereby providing options to streamline a wide variety of workflows for medium throughput high volume sample preparation methodologies depending on filtration.

For example, centrifugation and filtration have been widely accepted as techniques required for clarifying complex cell cultures to recover extracellular proteins such as monoclonal antibodies (mABs). These steps can be time consuming and costly for labs growing their cultures in 24-well culture plates. Use of filter plates streamline clarification of mammalian cell cultures when compared to traditional methods such as centrifugation/filtration/flocculation. We describe assessment of Pall's AcroPrep™ 24-well clarification and sterilization plate and AcroPrep 24-well sterilization plate for clarification of mammalian cell cultures (2 to 26 x 10^6 cells/mL) and recovery of proteins present in the supernatant. In addition, properties of 24-well plates for ultrafiltration (molecular weight cut offs ranging from 1 to 100 kDa), microfiltration (pore sizes ranging from 0.1 to 5 µm) and macrofiltration/particle filtration (30-40 µm non-woven media) membranes are described.

METHODS

Pall Filter Plates

- AcroPrep 24-well Clarification and Sterile Filtration plate with depth filter plus 0.65/0.2 µm Supor® EKV membrane
- AcroPrep 24-well Sterile Filtration plate with 0.65/0.2 µm Supor EKV membrane
- AcroPrep 24-well plates with Omega™ membrane for ultrafiltration with MWCOs of 1K, 3K, 10K, 30K, 50K, and 100K.
- AcroPrep 24-well plates with Supor PES membrane for microfiltration with pore sizes of 0.1, 0.45, 0.8, 1.2, and 5 µm.

Filtration Conditions

Table 1. Processing conditions

Property	Ultrafiltration	Microfiltration
Membrane MWCO (kDa) or pore size (µm)	1, 3, 10, 30, 50, 70, 100	0.1, 0.45, 0.8, 1.2, 5
Solute	Protein	Latex beads
Volume (mL/well)	4	3
Centrifugal force (x g)	1,500	1,500
Vacuum (inHg)	15	15
Positive Pressure (psi)	50	20

The AcroPrep 24-well Clarification and Sterile Filtration plate and the AcroPrep 24-well Sterile Filtration plate were processed by centrifugation at 1,000 x g or by vacuum at 15 inHg.

Cell Culture

CHO cells were cultured in Gibco◆ Dynamis◆ medium (Thermo Fisher Scientific) supplemented with 0.87 g/L of UltraGlutamine◆ I Supplement (Lonza) and puromycin (1.25 mL/L), at 37 °C, 135 rpm, and 8% CO_2. Cells were harvested at a density of 2-3 x 10^6 cells/mL and a viability > 98%. For clarification with the AcroPrep 24-well Clarification and Sterile Filtration plate culture, a suspension of ≥26 x 10^6 cells/mL was prepared from a culture of 2-3 x 10^6 cells/mL by centrifugation at 1,000 x g for 10 min and subsequent removal of an appropriate volume of supernatant. The cells were resuspended to a final density of 2.6 x 10^7 cells/mL. HEK293T cells were cultured in FreeStyle◆ 293 Expression Medium (Thermo Fisher Scientific) supplemented with 0.1% of Pluronic◆ F 68 non-ionic surfactant (Thermo Fisher Scientific) at 37 °C, 135 rpm, and 5% CO_2. At harvesting, the cultures had a density of 2-4 x 10^6 cells/mL and a viability >98%. For a sterile filtration experiment with the AcroPrep 24-well Sterile Filtration plate, a culture with a density of 4 x 10^6 cells/mL and >98% viability was first clarified by centrifugation at 700 x g for 5 min. Turbidity, pH, and protein recovery were assessed before and after filtration of the cell-free extract.

Hold-up volume, Processing Time, pH, Conductivity, Turbidity, Optical Density

The hold-up volume (sample volume that cannot be recovered after filtration), was determined as the difference between the recovered filtrate volume and the input volume of CHO cell culture (5 mL/well). Processing times of filtration of mammalian cultures under centrifugation and vacuum were determined by measuring the time for the culture aliquots to filter through the plate. Experiments were carried out with 5 mL/well aliquots of a high-density CHO cell culture whereas for HEK293T cells 6 and 7 mL/well aliquots were used for centrifugation and vacuum, respectively. Before and after filtration, pH and conductivity of the mammalian cell cultures were measured for several wells in various plates. To provide an indication of the removal of cells and cell debris by the filtration step, turbidity and optical density (OD) at 600 nm wavelength of the mammalian cell culture were also determined before and after filtration. The raw culture prior to filtration was first diluted in growth media before measuring turbidity and OD. The filtered samples were used undiluted but required pooling of several wells to obtain 10 mL samples for turbidity measurements at 600 nm.

Protein Recovery

To determine protein recovery, an aliquot of the initial mammalian cell culture was spiked with Gamma-globulin (IgG). Recovery was determined for total protein as measured with an Invitrogen◆ Qubit◆ 4 Fluorometer (Thermo Fisher Scientific) using the Qubit Protein Assay Kit (Thermo Fisher Scientific) according to the manufacturer's instructions and also for the IgG fraction specifically as measured by label-free bio-layer interferometry (BLI) on a FortéBio◆ Octet◆ instrument (Sartorius) with protein A Biosensors. This required measuring both parameters in the filtrate and in the initial spiked culture. To avoid interference with the detection methods in the spiked culture, cells were removed from a small volume of concentrated culture by centrifugation at 1,000 x g for 5 minutes, with or without filtration through a 0.2 µm Nylon filter, or alternatively through addition of a small amount of diatomaceous earth to a small volume of concentrated CHO cell culture followed by centrifugation at 1,000 x g for 5 minutes and/or filtration though a 0.2 µm filter.

Latex Bead Retention Test

Aliquots (3 mL/well) of 0.05% latex bead suspensions in 0.01% Tween◆ 20 were loaded into the filter plate. Filtration took place by centrifugation, vacuum, or positive pressure according to conditions described in table until all the upstream solution had filtered through. The percentage of latex beads retained on the filters (retention) was obtained by comparing the corrected absorbances at 260 nm of the input solution prior to filtration and of the filtrate (downstream). Due to the quick precipitation of the 100 µm diameter latex beads, concentration and the retention for each filtrate had to be estimated by visual comparison to a standard curve for those beads.

Protein Retention Test

Protein solutions were prepared at 1 mg/mL in 1X PBS buffer. Aliquots (4 mL/well) of the proteins were loaded into the filter plate after which they were processed by centrifugation, vacuum or positive pressure according to the condition described in Table 1. The percentage of protein retained by the filter plate was obtained by comparing the corrected absorbances at 280 nm of the input solution prior to (upstream) filtration and the filtrate in the collection plate after filtration (downstream).

SDS-PAGE

Filter plates with 30K and 50K MWCO were loaded with 0.3-1 mL/well aliquots of the indicated proteins at 1 mg/mL concentration. Individual wells of a 30K MWCO filter plate received samples of Myoglobulin (17.8 kDa), BSA (66 kDa), transferrin (79.5 kDa), and a mixture of the three proteins. The 50K MWCO plate received samples of Myoglobulin, Transferrin, IgG (150 kDa), and a mixture of the latter three proteins. Filtration took place by centrifugation at 1,500 x g for 10 min. Small aliquots of the input material, filtrate, and where indicated the retentate were diluted and mixed with loading buffer. Following a heating step at 70 °C for 10min, 20 µL aliquots were loaded onto a 4-12% Bis-Tris SDS-PAGE with 1X NuPAGE MOPS SDS running buffer. The resulting gels were then stained with Blue BANDit◆ protein stain until appearance of the bands and followed by a water rinse.

RESULTS

Processing of Cell Cultures with Clarification and Sterile Filtration Plates

Table 2. Processing of CHO cells

Plate	N.A.	Clarification and Sterile Filtration	
Input	Initial raw culture	Initial raw culture	
Density (cells/mL)	26 x 10^6	26 x 10^6	
Filtrate	N.A.	Clarified culture	
Processing Method	N.A.	Vacuum	Centrifuge
Sample Volume (mL/well)	N.A.	5	5
Processing time (min)	N.A.	20.2 ± 6.3	15.0
Hold-up volume (µL/well)	N.A.	300 - 450	400 - 450
pH	7.2	7.3	6.8
Conductivity (µS/cm)	10,100	9,200	9,700
Turbidity (NTU)	1,900 – 2,600	1.8	2.4
OD_{600}	18-19	0	0
Total Protein Recovery (%)	N.A.	98.3 ± 8.2	95.4 ± 11.4
IgG Recovery (%)	N.A.	91.3 ± 11	85.0 ±6.9

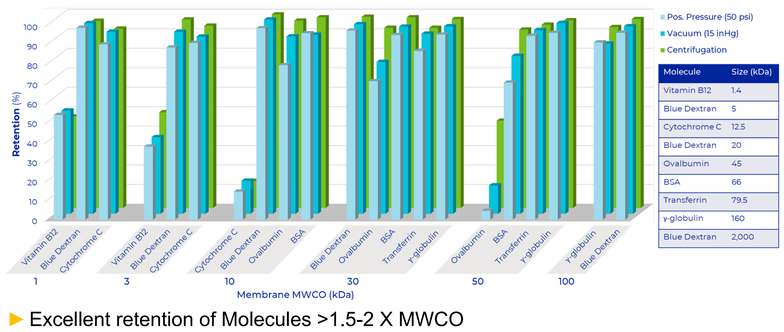
Table 3. Processing of HEK293T cells

Plate	N.A.	Clarification and Sterile Filtration	Sterile Filtration	
Input	Initial raw culture	Initial raw culture	Clarified Supernatant	
Density (cells/mL)	2-4 x 10^6	2 x 10^6	4 x 10^{6*}	
Filtrate	N.A.	Clarified culture	Filtered supernatant	
Processing Method	N.A.	Vacuum	Vacuum	Centrifuge
Sample Volume (mL/well)	N.A.	7	7	6
Processing time (min)	N.A.	4.3 ± 0.4	2.7 ± 0.7	5.0
pH	7.1 – 7.6	7.2		
Conductivity (µS/cm)	10,836	10,906		
Turbidity (NTU)	1,900 – 2,600	0.91		
OD_{600}	1.3 – 2.0	0.001		
Total Protein Recovery (%)	N.A.	101.2 ± 0.4	101.2 ± 1.0	99.4 ± 1.0

- Efficient removal of mammalian cells and cell debris
- Excellent protein recovery

Ultrafiltration

Figure 1. Retention by ultrafiltration plates of samples processed by centrifuge, vacuum, and positive pressure



- Excellent retention of Molecules >1.5-2 X MWCO

Protein Retention of Plates with 30K and 50K MWCO

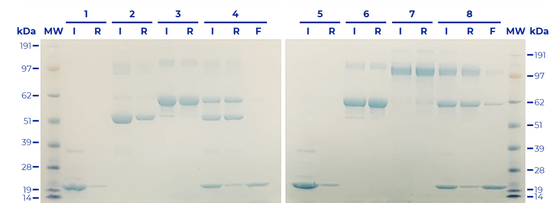
Figure 2. Protein retention (%) determined from absorbances at 280 nm (top panels) and visualization by SDS-PAGE (bottom panels) of proteins before (I) and after (R and F for retentate and filtrate, respectively) filtration with 30K (left panels) and 50K (right panels) MWCO filter plates.

30 K MWCO

Protein	MW (kDa)	Retention (%)
Myoglobulin	17.8	~0
BSA	66	98.0 ± 0.3
Transferrin	79.5	92.6 ± 1.4

50 K MWCO

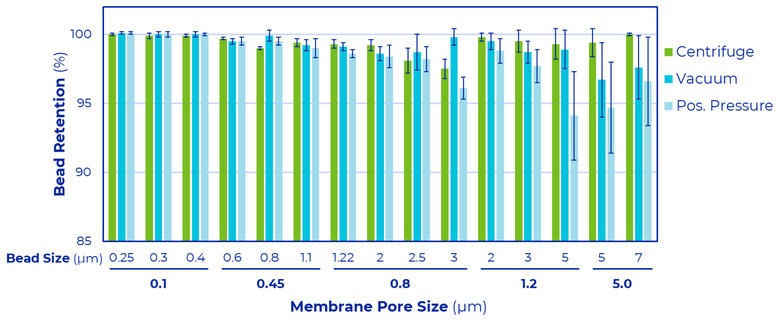
Protein	MW (kDa)	Retention (%)
Myoglobulin	17.8	~0
Transferrin	79.5	94.2 ± 2.4
IgG	150	96.1 ± 1.1



- 1 and 5 - Myoglobulin; 2 - BSA; 3 and 6 - Transferrin; 4 - Myoglobulin + BSA + Transferrin; 7 - IgG; 8 - Myoglobulin + BSA + IgG
- Excellent retention of Molecules >1.5-2 X MWCO

Latex Bead Retention of Microfiltration Plates

Figure 3. Retention by microfiltration plates of latex bead suspensions in following filtration by centrifugation, under vacuum, and under positive pressure



- Size of latex beads used to challenge the plates ranged from 1.5 to 5 times the membrane pore size
- All plates retained >94% of latex beads with similar efficiency for centrifuge, vacuum, and positive pressure
- The quick precipitation of the 100 µm diameter latex beads prevented accurate absorbance measurements. Retention for those filtrates with 30-40 µm filter plates was estimated by visual comparison to a standard curve for those beads (data not presented).

CONCLUSIONS

- 24-well filter plates with a broad range of filter media options that complement each other well and allow integration in complete workflow
- Single plate that combines cell clarification and sterile grade filtration and provides excellent protein recovery