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Application Note

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Concentration of Mammalian Cell Culture Supernatants with Vivaspin® Turbo 15 PES and Amicon® Ultra-15 Devices

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Abstract

Mammalian cell culture molecule separation processes commonly rely on concentration methods to provide molecule of interest concentration values, that are needed for various downstream processes, such as western blot and mass spectrometry analysis. Ultrafiltration techniques enable rapid and precise concentration across a range of volumes, but can incur loss of yield if sub-optimal membrane, molecular weight cut off (MWCO) or device protocols are employed. Here we benchmark two different membranes and MWCO devices for the concentration of a molecule of interest: Trefoil factor 1, to demonstrate the resulting differences in yield percentage recovery.

Summary

Downstream processing of Mammalian cell cultures has become increasingly critical as more laboratories move over to mammalian cell culture platforms for their recombinant protein expression. Typically a native, transient or stable cell line is generated where the protein of interest is secreted into the medium during a short period of time (from 1 to 48 hours). Multiple cell lines are often generated in parallel and tested under differing growth and expression conditions by culturing in a small well or flask. This increases efficiency by reducing time and material costs. Unfortunately, due to the low cell numbers and short expression times the secreted protein often makes up only a small proportion of the total protein content of the media. Processing of the conditioned media requires efficient high recovery ultrafiltration to provide sufficient concentrated protein for identification via various downstream processes (for example SDS polyacrylamide gel electrophoresis, Western blot, and mass spectrometry analysis). Thus enabling multiple cell lines, growth, and expression conditions to be assess in parallel both quickly and accurately prior to expensive scaling up.

Wherever possible, the smallest molecular weight cut off (MWCO) size should be used to prevent losses of low molecular weight proteins fragments. However, due to the complex nature of mammalian cell culture supernatants ultrafiltration devices can often fail resulting in high losses and low recovery rates (< 5%) leaving little or no available sample for analysis.

Sartorius's Vivaspin® Turbo 15 PES ultrafiltration devices utilize a high recovery polyethersulfone membrane in comparison to Millipore's Amicon® Ultra-15 devices which utilize a regenerated cellulose membrane. Both combine the latest ultrafiltration technology with low binding materials to reduce centrifugation time and increase recovery rates with high chemical compatibility. These are critical requirements for ultrafiltration of complex samples such as mammalian cell culture supernatants. This note compares the Sartorius's Vivaspin® Turbo 15 PES with Millipore's Amicon® Ultra-15 for media concentration efficiency.

Suggested Method

1. Select a Vivaspin® Turbo 15 PES or Amicon® Ultra-15 with a low MWCO (3,000 or 5,000 kDa) size for maximum sample recovery[1].
2. Fill the Vivaspin® Turbo 15 PES or Amicon® Ultra-15 with up to 15 mL media volume ensuring the screw closure is fully sealed.
3. Centrifuge for the recommended amount of time at an appropriate speed for your MWCO membrane and centrifuge type (swing or fixed rotor).
4. Empty the filtrate container[2] and refill the concentrator with additional sample if required.
5. Centrifuge again as before (repeat until entire sample has been loaded)[3].
6. Centrifuge until sample reaches the desired volume (typically 0.2 – 1 mL).
7. Recover the concentrate from the insert with a pipette.

Notes

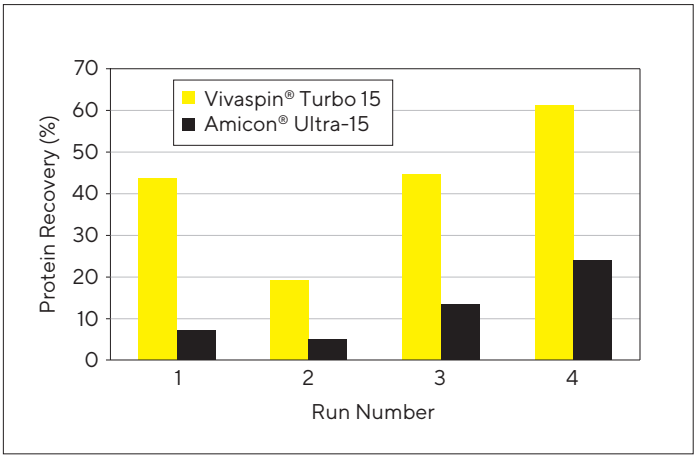
[1] For some downstream trial applications it may be necessary to pre-clean the membrane with ultrapure water prior to use.

[2] Filtrate volumes should be retained until the concentrated sample has been analysed.

[3] For downstream processes which have defined solvent requirements refill the concentrator with an appropriate solvent and centrifuge again as before.

Results and Discussion

As an example, the effect of mammalian cell culture concentration was analysed for downstream Western blot analysis. An OE19 Oesophagus | gastric cardia cell line was grown to confluence in a Nunc™ T75 flask before incubating for 24 hours in 5 mL of serum free Roswell Park Memorial Institute (RPMI) media containing l-glutamine additive. The media containing the secreted protein was removed and centrifuged to remove cell debris before pooling and concentrating using either a Vivaspin® Turbo 15 PES or Amicon® Ultra-15. The total start and final protein content was calculated using the Pierce™ BCA Protein Assay Kit and a NanoDrop spectrophotometer. The Vivaspin® Turbo 15 PES showed successful ultrafiltration giving a maximal recovery rate of 61.1%, with all runs providing sufficient recovery for successful downstream identification via Western blot analysis with the appropriate antibody. In comparison the Amicon® Ultra-15 showed 4 times lower protein recovery with a 50% failure rate (on downstream identification) and maximum recovery of 23.8%. Optimum recovery was seen using a fixed angle rotor with the insert | membrane directed perpendicular to the direction of rotation (Table 1 and Graph 1).



Graph 1: Protein recovery percentage with Vivaspin® Turbo 15 PES and the Amicon® Ultra-15 over 4 parallel runs.

	Run 1		Run 2	
Device	Vivaspin® Turbo 15 PES	Amicon® Ultra-15	Vivaspin® Turbo 15 PES	Amicon® Ultra-15
MWCO	3 kDa	3 kDa	3 kDa	3 kDa
Start volume (mL)	15.01	14.98	14.92	14.91
Final volume (mL)	0.72	0.57	0.64	1.08
Centrifuge type	Swing bucket	Swing bucket	Swing bucket	Swing bucket
Total centrifugation time (min)	60	60	60	60
Times concentrated	20.8	26.3	23.3	13.8
Percentage recovery (%)	43.4	6.9	19.1	4.9

	Run 3		Run 4	
Device	Vivaspin® Turbo 15 PES	Amicon® Ultra-15	Vivaspin® Turbo 15 PES	Amicon® Ultra-15
MWCO	5 kDa	3 kDa	5 kDa	3 kDa
Start volume (mL)	15.39	14.68	15.00	14.82
Final volume (mL)	0.29	0.55	0.18	0.50
Centrifuge type	fixed angle	fixed angle	fixed angle	fixed angle
Total centrifugation time (min)	130	330	130	330
Times concentrated	53.1	26.7	83.3	29.6
Percentage recovery (%)	44.5	13.1	61.1	23.8

Table 1: Protein recovery details from 4 parallel runs using the Vivaspin® Turbo 15 PES (polyethersulfone membrane) and the Amicon® Ultra-15 (regenerated cellulose membrane).

Test sample

- Cell line: OE19 Oesophagus | gastric cardia from human source
- Media: serum free Roswell Park Memorial Institute (RPMI) with l-glutamine additive
- Protein of interest: Trefoil factor 1 (TFF1)
- Size of protein: 6.67 kDa
- Test site: Northern Institute for Cancer Research, Medical School, Newcastle University
- Commissioned by: Generon Ltd, 12 Rawcliffe House, Howarth Road, Maidenhead

Equipment

- Sartorius Vivaspin® Turbo 15 PES, 3,000 kDa MWCO
- Sartorius Vivaspin® Turbo 15 PES, 5,000 kDa MWCO
- Millipore Amicon® Ultra-15, 3,000 kDa MWCO
- Nunc™ T75 flask
- Pierce™ BCA Protein Assay Kit
- Mettler Toledo PB1502-S balance
- Beckman Coulter Allegra X-12R centrifuge with swing angle rotor
- Beckman Coulter Allegra 25R with fixed angle rotor
- Thermo Scientific NanoDrop spectrophotometer
- Standard Gilson pipettes and tips

Testimonial

"We found the Vivaspin® Turbo's consistently 4 times more efficient and easier to use than the Amicon® Ultra-15's. It's stopped reservoir and slanted design were very effective at concentrating my protein of interest as verified by Western blot and we will be using these devices for all our complex media concentration applications in the future." – Dr Joseph Willet, Newcastle University.

Each ultrafiltration device was tested with 15 mL of serum free RPMI media containing l-glutamine additive and the secreted TFF1 protein from OE19 Oesophagus | gastric cardia cell line incubated for 24 hours. Each centrifugation was performed at 3,000 g (swing bucket) or 4,000 g (fixed angle) depending upon the centrifuge type. Accumulative centrifugation times are shown. Protein concentration was measured at a wavelength of 562 nm using the Pierce™ BCA Protein Assay Kit and a Thermo Scientific NanoDrop spectrophotometer.

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