SARTORIUS

Frequently Asked Questions

Laboratory Ultrafiltration

1. What's the best way to concentrate large sample volumes in one go?

Concentrating medium to high volume samples can be time-consuming and costly in the laboratory setting. Happily, there are some good solutions for concentrating up to 5L of sample in the lab setting, without having to resort to the high-priced process scale systems that require large mounting systems and high capex investment. The **Vivaflow®** cross flow devices are the most robust, lab scale dedicated devices and come in two membrane options: PES and Hydrosart® (a patented Regenerated Cellulose membrane). There are both single use **(Vivaflow® 50**) and multi-use **(Vivaflow® 50R and Vivaflow® 200)** options to meet both economical and contamination prevention requirements. The high surface area, thin channel, flip flow recirculation design results in 50x concentration factors of 1L samples in just 30 minutes. Greater sample volumes such as 5L can be similarly concentrated in under 75 minutes. Near total recovery of the concentrate is achieved with a single final rinse.

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2. How can you reduce protein degradation during concentration?

Unbalanced protein to pH/sample buffer conditions and high shear stresses can commonly cause degradation during concentration. Likewise, the more linear the protein shape the greater the adverse effects on structure caused by high g-forces. If the protein is linear having a slower concentration period with lower g-forces than the maximum recommended (~50% the recommended is a good starting place) will help. For more globular molecules it is good practice to use a device closest to the minimum sample volume, this ensures a higher surface area and reduces the potential for blockage and increasing stress on the samples. Although it should be noted that with higher surface areas it increases the rate of non-specific binding if you have a non-optimal protein-membrane combination or if your protein is considered to be "sticky". Laboratory-based crossflow cassettes such as the **Vivaflow®** devices, offer a true parallel flow path for samples, further ensuring minimal shear stress. It should be noted that typically stirred cell vessels put on relatively high amounts of shear stress. For pH/buffer conditions, once the correct buffer ratio is selected it can be maintained using continuous buffer exchange techniques (via diafiltration). Buffer exchange can easily be carried out with the **Vivaflow®** devices and the centrifugal **Vivaspin® 20** and ensure that even with increasing concentrations the correct buffer balance is maintained, or a new buffer added if needed.

3. What ultrafiltration method is best suited for virus concentration?

Viral vectors and vaccine applications are a growing requirement in regenerative medicine. In general the principles of device, membrane and MWCO selection are the same as with proteins and other biomolecules, unless there are specific considerations for your virus. The rule of thumb is to choose a molecular weight cut off (MWCO) close to one third the molecular weight of your target. In the case of viruses where molecular weight is not as relevant as diameter there is a comparison chart which gives the best MWCO for diameter and DNA length (see Table A.). For Lentivirus for example which has a ~90nm diameter we find that 300K MWCO membranes work optimally, with 100K MWCOs also giving good results at the sacrifice of speed. Further more, membrane properties may have an impact also. Hydrosart® and Regenerated Cellulose have a neutral charge at pH 7, whereas PES has a slight negative charge which should be considered. In summary, testing and qualifying should always be undertaken. As an example, a recent investigation has shown that for optimal concentration of Lentivirus in a 15ml sample sample, a cross flow system (Ambr® Crossflow) with a 300K MWCO Hydrosart® membrane was best for recovery, closely followed by a 100K MWCO PES membrane.
The below link provides a review and further examples of optimal methods for virus concentration.

Learn More

4. How can I maximize my protein concentration and protein recovery?

Adjusting your process to your sample should be a priority, as the same membrane, MWCO and device combination may not be optimum for each protein class or even species. The following steps should be first taken when selecting your process; 1) Consider the sample and molecule properties; changes to pH can increase conformational rearrangements, lower temperatures can reduce concentration rates, etc. 2) Pick the right membrane, ultrafiltration is known for not having many membrane options, but you should test whether regenerated cellulose (RC), Hydrosart® or Polyethersulfone (PES) material provides the lowest non-specific binding and subsequently recovery, of your specific material. 3) Select the right MWCO, typically a MWCO 1/3 the size of the target is best for maximizing concentration, suppliers that advertise concentration of a typical test molecule close to a membrane MWCO (e.g. 12.4kDa Cytochrome-C with a 10K MWCO) are not considering "real life" proteins that may not behave as typically as Cytochrome-C. 4) Select the best suited device, Sartorius has the widest range of devices for low concentration samples, DNA, protein, virus, filtration applications, etc, selecting which is best suited can improve results . 5) Use appropriate device treatment methods, such as pre-rinsing to remove analytes or flushing with a non-interfering protein to passivate the binding sites and reduce loss.
Finally, 5) Consider sample control methods, such as using dead stops within devices to pipette out all the retentate, or pre-filling the filtrate tube to control the final volume of the retentate.

MWCO	Protein MW	Molecule Size	BPCO (dsDNA)	BPCO (ssDNA)	Estimated Pore Size
1,000 K	>3000 kDa	300 – 600 nm	>5000 bp	>9000 sb	100 nm
300 K	900 – 1800 kDa	90 – 200 nm	>1500 bp	>2900 sb	30 nm
100 K	300 – 900 kDa	30 – 90 nm	>600 bp	>900 sb	10 nm
50 K	150 – 300 kDa	15 – 30 nm	>300 bp	>475 sb	7 nm
30 K	90 – 180 kDa	9 – 15 nm	>50 bp	>275 sb	4 nm
10 K	30 – 90 kDa	5 – 9 nm	>30 bp	>90 sb	2.5 nm
5 K	15 – 30 kDa	3 – 5 nm	>20 bp	>50 sb	1.5 nm
3 K	10 – 20 kDa	2.5 – 3.6 nm	>15 bp	>30 sb	1.2 nm
2 K	3 – 10 kDa	2 – 3 nm	>10 bp	>10 sb	1 nm

Table A: Optimum Ultrafiltration Membrane MWCO with Correlating Molecule Size , Length or Molecular Weight

5. Can the Vivaspin® devices be spun to dryness?

No, all Vivaspin® devices have built in deadstops, ranging from 5uL to 100uL. So be sure to select the device with the right deadstop for your sample. Further, the Vivaspin® Turbo range has an angular deadstop to ensure every last uL can be aspirated.

6. Can Vivaspin®, Vivacell® and Vivaflow® devices be sterilized?

All devices can be treated with 70% ethanol and Ethylene Oxide (EtO) for gas sterilization. However note that no studies have been carried out to determine sterilization after treatment. For DNA studies, the Vivacon® PCR grade range has been EtO treated to deactivate any interferring trace DNA.

7. What membranes are available with the Sartorius ultrafiltration range?

We have a unique range of four ultrafiltration membranes to choose from. Regenerated Cellulose (RC) for general applications, polyethersulfone (PES) for maximising recoveries with some sample types, Cellulose Triacetate (CTA) generally for permeate/filtrate applications and our patented Hydrosart® membrane, a more chemically robust version of RC.

8. How should I select the most optimum MWCO?

Typically choose a MWCO 1/3 the size of the target molecule MW. Supplier options advertising 1/2 may have a pore matrix that is too “tight”, those advertising 1/6 may be too “loose”. Sartorius membranes have a porosity specified to offer the the best concentration values for all sample types, in combination with our widest range of membrane materials.

9. What is the chemical compatibility like with the Viva range?

Sartorius Lab Ultrafiltration devices are primarily intended for biological samples, but can be used for environmental and industrial samples. Compatibilities of housing and membrane material must be considered. Please refer to specific device Instructions For Use or Sartorius technical support. Typically the polypropylene based devices, such as the Vivaspin® Turbo range, offer the greatest chemical compatibility.

10. Can the Vivaspin®, Vivacell® and Vivaflow® devices be used for DNA?

All devices are able to concentrate double stranded and single stranded DNA, based on strand length. However for optimal recovery, please use Sartorius Vivacon® range. Specially designed with a horizontal Hydrosart® membrane for dilute DNA samples. Reverse centrifugation and PCR grade devices are available.

11. What volumes ranges are devices available in?

Lab based devices are available for concentration of sample volumes from 100uLto 5L. Specifically; Vivaspin® 500 100-500uL, Vivaspin® 2 0.4-2mL, Vivaspin® Turbo 4 2-4mL, Vivaspin® 2-6mL, Vivaspin® 15R 4-15mL, Vivaspin® Turbo 15 4-15mL, Vivaspin® 20 5-20mL, Vivacell® 100 20-100mL, Vivaflow® 50 50mL - 3L, Vivaflow® 50R 50mL - 1L, Vivaflow® 200 0.5-5L.

12. Are application guides available for my more niche requirements?

Sartorius Lab Ultrafiltration offers the widest range of lab based ultrafiltration application guides. These can be found at the below link, further guides are available by contacting Sartorius technical support:

Learn More

Find further details of all tips, tricks, applications and products by contacting your local Sartorius representative.

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