

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 dedicated solutions for concentrating samples with initial volumes in the 0.1 - 5 L range, without having to resort to the process scale systems that require large mounting systems and high CAPEX investment. **Vivaflow**[®] cross flow devices are the most robust, lab scale dedicated devices and are offered with two membrane options: PES or Hydrosart[®]. There is a choice of single use (**Vivaflow**[®] **50**) or multi-use (**Vivaflow**[®] **50R and 200**) options to meet both economical and contamination prevention requirements. The high surface area, thin channel, flip flow recirculation design results in 50x concentration of 1L samples in just 30 minutes. Similarly, initial sample volumes up to 5 L can be concentrated in under 75 minutes. Near total recovery of the concentrate is achieved with a single buffer rinse.

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2. How can I reduce protein degradation during concentration?

Incorrect buffer compositions 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 relative centrifugal forces. In such cases, reducing the centrifuge speed to around half of the maximum RCF is a good starting point for process optimization. For globular molecules which are prone to aggregation, it is good practice to use a device which has a minimum sample capacity close to your starting volume. This generally ensures a higher membrane surface area, reducing the potential for blockages which can contribute to increased stress on the sample. Note, however, that the increased membrane area also represents an increase in the overall internal surface area of the device which is in contact with the sample, both of which can result in higher non-specific adsorption. Non-specific adsorption may also be more pronounced where the membrane material in use is not the optimal choice for the target molecule, or where the target is considered to be "sticky". Laboratory crossflow cassettes, such as **Vivaflow**[®], feature flow paths which carry the sample parallel to the membrane, further minimizing shear stresses in comparison to centrifugal devices. It should be noted that conventional stirred cell devices apply relatively high shear stresses on the sample. Finally, when the optimal buffer conditions (pH and composition) have been determined, these can be maintained or adjusted during concentration, using diafiltration for sequential or simultaneous buffer exchange or desalting. Buffer exchange is simplified with the **Vivaflow**[®] diafiltration reservoir and **Vivaspin**[®] **20** diafiltration cups, which ensure that even with increasing target molecule concentrations, the correct buffer balance is maintained or adjusted, as needed.

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

Viruses and viral vectors are in growing demand in therapeutics, vaccine and regenerative medicine applications. 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 viral target. 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 diameter is a more relevant measure of size, we provide a handy table to help you find the most appropriate MWCO (see Table A.). For example, concentration time and recovery for Lentivirus, with a diameter of ~90 nm, is typically optimal with a 300 kDa MWCO ultrafiltration membrane. A 100 kDa MWCO also provides good results, though with a compromise on process speed. Membrane properties should also be considered, as they may have an impact on recoveries. For instance, Hydrosart® and regenerated cellulose (RC) have no net charge at pH 7, whereas PES has a slight negative charge. These MWCO and membrane material considerations have been tested at Sartorius using Ambr® Crossflow. The results showed that optimal concentration of Lentivirus from a 15 mL sample was achieved with a 300 kDa MWCO Hydrosart® membrane, closely followed by 100 kDa PES. It is good practice to test and qualify the device, membrane and MWCO independently for each target molecule.

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

Designing an ultrafiltration process to suit your specific sample type should be a priority, as the same membrane, MWCO and device combination may not be optimal for each protein type or class. The following parameters should be considered; 1) Sample and molecule properties; changes to pH can increase conformational rearrangements, lower temperatures can reduce concentration rates, etc. 2) Membrane material; ultrafiltration may not have such a broad choice of membrane materials as microfiltration but it is none-the-less important to test whether PES, RC, CTA or HY will provide the lowest non-specific binding and subsequently highest target recoveries. 3) MWCO; remember that molecules used to illustrate device performance (e.g. cytochrome c (12.4 kDa) with a 10 kDa MWCO) are not usually representative of “real-world” target proteins, choosing a MWCO around 1/3 the size of the target will typically offer the best performance. 4) Device design; Sartorius offers the widest range of devices, with some options dedicated to specific sample types and applications, such as samples with low initial concentrations, nucleic acids, proteins, viruses and filtrate analyses. 5) Device pre-treatment; pre-rinsing to remove analytes or flushing with a non-interfering protein to passivate non-specific binding sites and reduce target molecule loss. Finally, 6) Control methods; use devices with an appropriate dead-stop pocket to ensure the entire retentate sample can be retrieved after concentration, pre-fill the filtrate tube to control the final retentate volume.

MWCO	Protein MW	Molecule Size	dsDNA Length	ssDNA Length	Estimated Pore Size
1,000 kDa	>3,000 kDa	300 – 600 nm	>5,000 bp	>9,000 sb	100 nm
300 kDa	900 – 1,800 kDa	90 – 200 nm	>1,500 bp	>2,900 sb	30 nm
100 kDa	300 – 900 kDa	30 – 90 nm	>600 bp	>900 sb	10 nm
50 kDa	150 – 300 kDa	15 – 30 nm	>300 bp	>475 sb	7 nm
30 kDa	90 – 180 kDa	9 – 15 nm	>50 bp	>275 sb	4 nm
10 kDa	30 – 90 kDa	5 – 9 nm	>30 bp	>90 sb	2.5 nm
5 kDa	15 – 30 kDa	3 – 5 nm	>20 bp	>50 sb	1.5 nm
3 kDa	10 – 20 kDa	2.5 – 3.6 nm	>15 bp	>30 sb	1.2 nm
2 kDa	3 – 10 kDa	2 – 3 nm	>10 bp	>10 sb	1 nm

Table A: Optimum ultrafiltration membrane MWCOs correlated to target molecule weight, size or length.

5. Is there a risk of concentrating samples to dryness with Vivaspin®?

No, all Vivaspin® devices have integral dead-stops, with minimum volumes ranging from 5 - 350 µL. This ensures the highest concentration factors can be reached without the possibility of drying out the sample. In addition, the Vivaspin® Turbo devices feature angular dead-stop pockets, which make retrieval of every last microlitre of the retentate sample even more straightforward.

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

All of these devices can be sanitized using 70% ethanol or with ethylene oxide (EO) gas treatment. However, no studies have been carried out to confirm sterility after such treatments. Where it is important that residual DNA does not interfere with subsequent processing or analysis, PCR Grade Vivacon® devices are available. These are treated in a validated EO process to inactivate trace DNA.

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

There is a unique and comprehensive range of four ultrafiltration membranes to choose from. Polyethersulfone (PES) and regenerated cellulose (RC) for general applications, cellulose triacetate (CTA) typically recommended for permeate | filtrate applications, and our patented Hydrosart® (HY) membrane, a more chemically robust variant of RC.

8. How should I select the optimum MWCO?

Typically choose a MWCO 1/3 the size of the target molecule MW. Options which recommend lower or higher MWCO selections typically feature pore matrices with looser or tighter structures, resulting in lower recoveries or slower processing speeds. Sartorius membranes have a porosity specified to offer the best of both worlds - high target molecule recoveries and shortest concentration times.

9. Will Vivaspin® and Vivaflow® be compatible with my sample?

Sartorius lab ultrafiltration devices are primarily intended for biological samples, but can be used in environmental and industrial applications. Compatibilities of housing and membrane material must be considered. The Instructions For Use for each device provides guidance on compatibility with some common laboratory reagents. Vivaspin® Turbo features more chemically resistant materials, which will typically offer broader chemical compatibility, when needed.

10. Can I concentrate nucleic acid samples?

All devices are able to concentrate double and single stranded nucleic acids, based on strand length. However, for optimal recovery, we recommend to use Vivacon® devices, which have been specially designed with a horizontal Hydrosart® membrane, are reverse spin enabled for pipette-free sample recovery, and offered in PCR grade versions to prevent unwanted interference.

11. What are the sample capacities for each device?

Lab ultrafiltration devices are available for the concentration of samples with an initial volume from 100 µL to 5 L.

- Vivaspin® 500: 100 - 500 µL
- Vivaspin® 2: 0.4 - 2 mL
- Vivaspin® Filtrate: 0.4 - 2.5 mL
- Vivaspin® Turbo 4: 2 - 4 mL
- Vivaspin® 6: 2 - 6 mL
- Vivaspin® 15R: 4 - 15 mL
- Vivaspin® Turbo 15: 4 - 15 mL
- Vivaspin® 20: 5 - 20 mL
- Vivaspin® 100: 20 - 100 mL
- Vivaflow® 50: 0.1 - 3 L
- Vivaflow® 50R: 0.1 - 1 L
- Vivaflow® 200: 0.5 - 5 L
- Vivacon® 500: 100 - 500 µL
- Vivacon® 2: 0.4 - 2 mL

12. Are application guides available for my requirements?

With the widest range of lab ultrafiltration application guides, there is sure to be a review or data to support your choice of the optimal device. Follow the link below or speak with your Sartorius contact for more information.

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