

Application Note

Amicon® Stirred Cell enables gentle and efficient large volume sample concentration and continuous diafiltration

Introduction

Sample preparation of macromolecule solutions, such as proteins, enzymes, antibodies, and viruses, often yield large volumes of diluted macrosolutes in buffers that are incompatible with downstream processes or detection. Centrifugal ultrafiltration devices (such as the Amicon® Ultra Filters) are regularly used to concentrate and buffer-exchange these types of macrosolutes; however, volumes larger than 50 mL present significant challenges, requiring samples to be loaded in multiple stages or aliquoted over several devices.

The Amicon® Stirred Cell family of pressure-driven filtration devices provide an ideal solution for concentrating and buffer-exchanging large volumes of macrosolutes. These devices are available in multiple sizes to offer a wide range of processing volumes. Additionally, to accommodate expansion to even larger processing volumes, an external reservoir can be attached to any Amicon® Stirred Cell. Unlike ultrafiltration with tangential flow filtration (TFF) devices, which are manufactured with pre-sealed membranes, the Amicon® Stirred Cells use membrane discs, which are easily exchanged to match changing sample preparation needs.



Amicon® Stirred Cells

Volume	Membrane Diameter
50 mL	44.5 mm
200 mL	63.5 mm
400 mL	76 mm

Table 1. Volume and membrane diameter of Amicon® Stirred Cells.

The new generation of Amicon® Stirred Cells provides ergonomic benefits, integrated safety features, a more secure stir bar, superior integrity, and ease of use while also providing a broader selection of membrane discs.

Simultaneous sample concentration and buffer exchange

Ultrafiltration is a proven method for sample concentration. When combined with diafiltration, the analyte of interest is provided at a concentration and in a buffer that is compatible with additional purification and analysis steps. During ultrafiltration, the desired macrosolute concentration is achieved because the concentration of non-permeating species is increased while the fluid volume is reduced. Furthermore, the concentration of membrane-permeating species such as salts and microsolute remains unchanged.

How to choose the correct ultrafiltration membrane for the sample

When using ultrafiltration for sample concentration, particular attention has to be paid in choosing the correct membrane molecular weight cut-off, as well as membrane material. Ultrafiltration membranes are typically made from regenerated cellulose or polyethersulfone (PES). The material of choice will greatly depend on sample compatibility. The choice of membrane nominal molecular weight cut-off (NMWCO) will depend on the molecular weight (MW) of the macrosolute that is to be retained. Membrane choice will have a significant impact on performance. As a rule, the NMWCO should be 2-3 times smaller than the molecular weight of the solute to be retained when using regenerated cellulose and 4-5 times smaller for Biomax® PES membranes. Solute retention may be further influenced by processing temperature, operating pressure, stir speed, sample concentration and sample constitution and may require

optimization to assure desired yield. The most frequently used ultrafiltration membranes have a NMWCO range of 3 kDa to 100 kDa, although smaller and larger pore sizes are available.

Diafiltration compared to dialysis

Diafiltration is a technique that uses ultrafiltration for buffer exchange. Diafiltration can rapidly and efficiently eliminate salts and/or microsolute from macromolecular mixtures. This process is typically called "washing out." The term "washing in" is used when diafiltration is employed to replace one salt species or microsolute with another, as is done during buffer exchange.

Traditional dialysis is an alternative buffer exchange technique; however, it has several drawbacks. It relies on slow diffusion and difficult-to-handle dialysis tubing or cassettes. In many cases, during the course of dialysis, the volume in the dialysis tubing increases as a consequence of osmosis, further diluting the sample and requiring a sample concentration step. Finally, dialysis can require large buffer volumes and multiple buffer changes.

In contrast, diafiltration uses ultrafiltration membranes, either in centrifugal or pressure-driven devices such as the Amicon® Stirred Cell for efficient buffer exchange. Unlike dialysis tubing, ultrafiltration membranes can be used for both sample concentration as well as buffer exchange, thus minimizing sample transfers and reducing sample loss. Furthermore, diafiltration often requires significantly less buffer volume than traditional dialysis.

Table 2. Comparison of diafiltration and dialysis

Diafiltration	Dialysis
Transport convective with solvents, independent of microsolite composition	Transport diffusion-controlled, dependent on type of microsolite
Smaller volume of exchange buffer required	High volume of exchange buffer volume required, efficient mixing and frequent buffer changes required to drive efficient transport
Rapid exchange rate. Fractional removal of solvent/microsolite is independent of sample composition	Exchange rate is slow and efficiency is reduced with decrease microsolite concentration
Ultrafiltration rate reduced with decreased temperature	Reduced microsolite transport with decreased temperature
At high macrosolute content, ultrafiltration rate and microsolite transport are reduced	Microsolite transport unaffected by content of retained macrosolutes
Scalable; compatible with samples ranging from few microliters to many liters	Not easily scalable to large volume, best used for microliter to liter volume samples.

Continuous vs. discontinuous diafiltration

Diafiltration can be performed in either continuous or discontinuous mode. Discontinuous diafiltration refers to a practice where the sample is first concentrated, then diluted with exchange buffer to the initial starting volume, followed by another concentration step. This process is repeated until the desired microsolutes have been exchanged. On the other hand, continuous diafiltration

maintains a constant volume throughout the buffer exchange process, by introducing exchange buffer at the same rate as filtrate is being removed. By maintaining a fixed volume, the retained solute concentration remains constant, providing a gentler method of buffer exchange. In general, continuous diafiltration is more efficient than discontinuous diafiltration. In discontinuous diafiltration, the permeate flux decreases as the sample concentration increases, which can contribute to membrane fouling and cause increased retention of microsolutes that would otherwise pass through the membrane.

Discontinuous Diafiltration

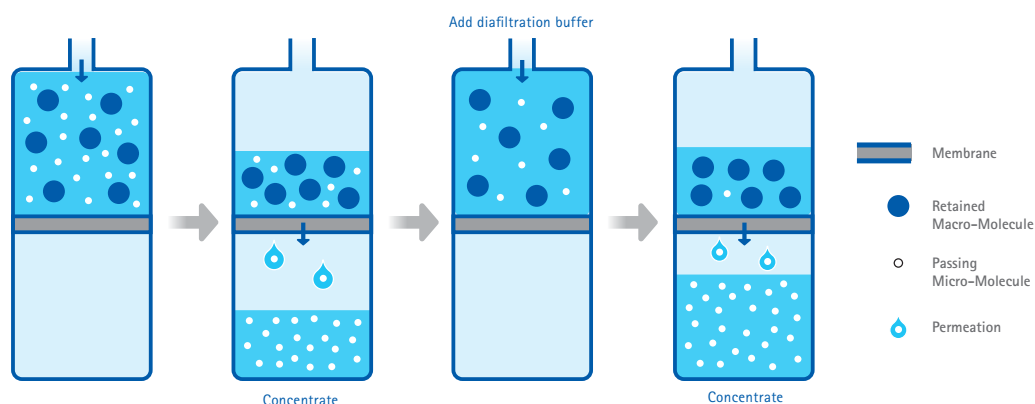
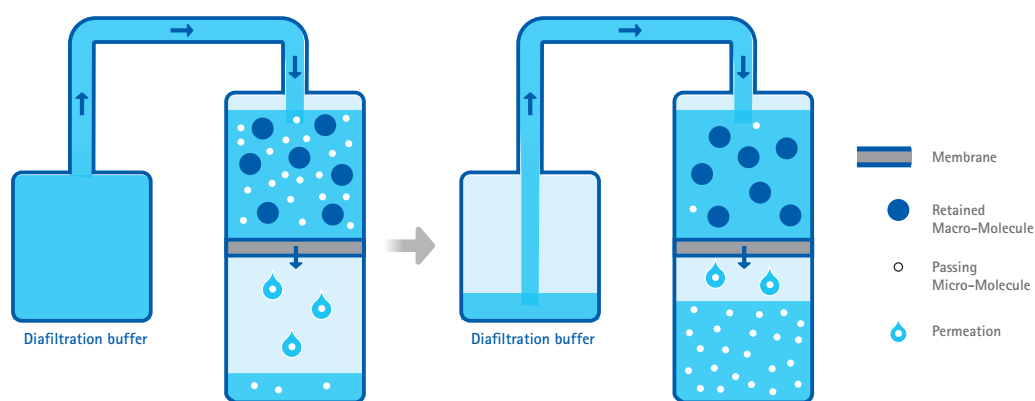


Figure 1. Comparison between discontinuous and continuous diafiltration for a solution containing a retained macrosolute and a passing microsolutes.

Continuous Diafiltration



Calculating buffer exchange volume for continuous diafiltration

The buffer requirement during diafiltration is typically expressed as "diafiltration volume" (DV). The DV is equal to the volume of the permeate removed during diafiltration divided by the volume of the remaining retentate (Equation 1).

[Equation 1]

$$DV = \frac{\text{volume of permeate removed}}{\text{volume of retentate remaining}}$$

Microsolute retention values (R) are partially retained by the membrane will require additional DV. As a general rule of thumb, using DV=three times the sample volume will wash out 95%, and using DV=five times the sample volume will remove 99% of a non-retained microsolutes. A more precise characterization for both wash-in and wash-out continuous diafiltration is illustrated in Figure 2. The figure demonstrates theoretical diafiltration volumes for microsolute retention values (R=0), as well as partially retained microsolute (R=0.1, 0.2 etc.). Equation 2 can be used for theoretical calculations of diafiltration volume requirements. However, these are guidelines and actual diafiltration volume requirements need to be empirically determined.

[Equation 2]

$$\text{Remaining contaminant } [\%] = 100 \times N \times e^{(R-1)}$$

where N = number of diafiltration volumes

and R = retention value of the microsolutes

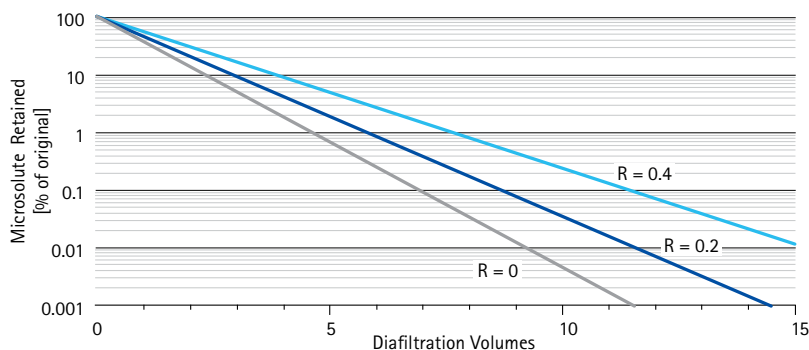


Figure 2. Effect of microsolute retention values (R) on exchange buffer volume requirement (DV) for removing microsolute by continuous diafiltration.

Calculating buffer exchange volume for discontinuous diafiltration

In discontinuous diafiltration, salt and microsolutes removal depend on the degree of concentration during the repeated concentration/dilution steps. The microsolutes level decreases according to Equation 3 during each step.

[Equation 3]

$$\frac{C}{C_0} = \frac{V_f}{V'_0}$$

Where C_0 = Initial microsolutes concentration

C = Concentration after the concentration and dilution steps

V_f = Sample volume after the concentration step

V'_0 = Sample volume after the concentration and dilution steps

Example: A 100 mL of a 1 mg/mL bovine serum albumin (BSA) protein sample containing 1 M salt is concentrated down to 50 mL, concentrating the protein to 2 mg/mL while the salt concentration is constant at 1 M. Then, the sample is diluted back to 100 mL, reducing BSA concentration to 1 mg/mL and the salt concentration down to 0.5 M. Each diafiltration step reduces the salt concentration by 50%. These two steps are repeated until the salt has been reduced to the desired concentration.

The salt concentration after each step can be calculated using Equations 3 and 4:

[Equation 4]

$$C = \frac{V_f \times C_0}{V'_0} = \frac{50 \text{ mL} \times 1 \text{ M}}{100 \text{ mL}} = 0.5 \text{ M}$$

The above should be viewed as a guideline, as sample types and composition vary greatly and over-concentration could have detrimental consequences.

While continuous diafiltration is traditionally used with large volume tangential flow filtration (TFF) systems, this gentle form of buffer exchange can be easily adopted for use with the Amicon® Stirred Cell. The same reservoir used to extend the processing volume during sample concentration can now be used for the diafiltration buffer. Once pressure has been applied and equalized throughout the setup, exchange buffer will enter the stirred cell at the same rate at which the permeate is being removed.

Materials and methods for large volume concentration and diafiltration with Amicon® Stirred Cells

Commonly, sample concentration is performed first to reduce the overall sample volume, followed by diafiltration. This approach significantly reduces the amount of diafiltration buffer required. However, if a sample is unstable or too viscous at higher concentration, a partial concentration may be performed first, followed by diafiltration. The final concentration step is then performed in the exchange buffer. This method will use more exchange buffer, but will maintain a greater permeate flux due to lower concentration or viscosity, reducing the process time and ultimately protecting sample integrity.

To demonstrate the utility of the Amicon® Stirred Cell for large volume concentration, a 10x concentration was performed, reducing 500 mL of a 0.1 mg/mL BSA solution with 1 M NaCl to a final volume of 50 mL. The experiment was performed using a 200 mL Amicon® Stirred Cell (cat. no. UFSC20001) with the 800 mL Amicon® Stirred Cell Reservoir (cat. no. 6028) to expand the total diafiltration volume range. Protein concentration and diafiltration were performed using a 10 kDa Regenerated Cellulose membrane (cat. no. PLGC06210). To enable quick and simple switching between concentration and diafiltration modes without interrupting system operation, the Amicon® Stirred Cell Selector Valve (cat. no. 6003) was installed between the external reservoir and the stirred cell (see continuous diafiltration setup below).

Large volume concentration

Following the user guide instructions for the selector valve, the inlet/outlet tube fittings were attached to the appropriate tubing. Then, both the Amicon® Stirred Cell and reservoir were assembled, and the reservoir was placed into the retaining stand. For 10x concentration of 500 mL of 0.1 mg/mL BSA in 1 M NaCl: 200 mL was added to the stirred cell and the remainder was added to the reservoir through the recessed sample port. The stirred cell was placed onto a magnetic stirrer.

To reduce hold-up volume, care should be taken to minimize tubing length. If necessary, the reservoir should be tilted toward the inlet tubing to assure that all the sample or buffer is transferred to the stirred cell during processing.

The selector valve was set to "Gas" mode (gas spool in) and stirring was initiated at 200 rpm. Then, nitrogen gas was applied at 50 psi, pressurizing the Amicon® Stirred Cell and the reservoir. The pressure was thus equalized over the liquid volume in both the stirred cell and the reservoir, allowing the sample to concentrate.

Once the BSA solution in the Amicon® Stirred Cell was concentrated to approximately 50 mL, the selector valve was switched to "Liquid" mode (liquid spool in). This allowed pressurized liquid to flow out of the reservoir and into the stirred cell. The liquid level in the stirred cell was maintained at about 50 mL during the process. The filtration was stopped when the filtrate reached 450 mL and the concentrate was at 50 mL (10x concentration). The pressure and magnetic stirring were turned off and pressure was vented from both devices. BSA concentration in the retentate and starting material were measured using A_{280nm} to assure that the final concentration of 1 mg/mL BSA was reached.

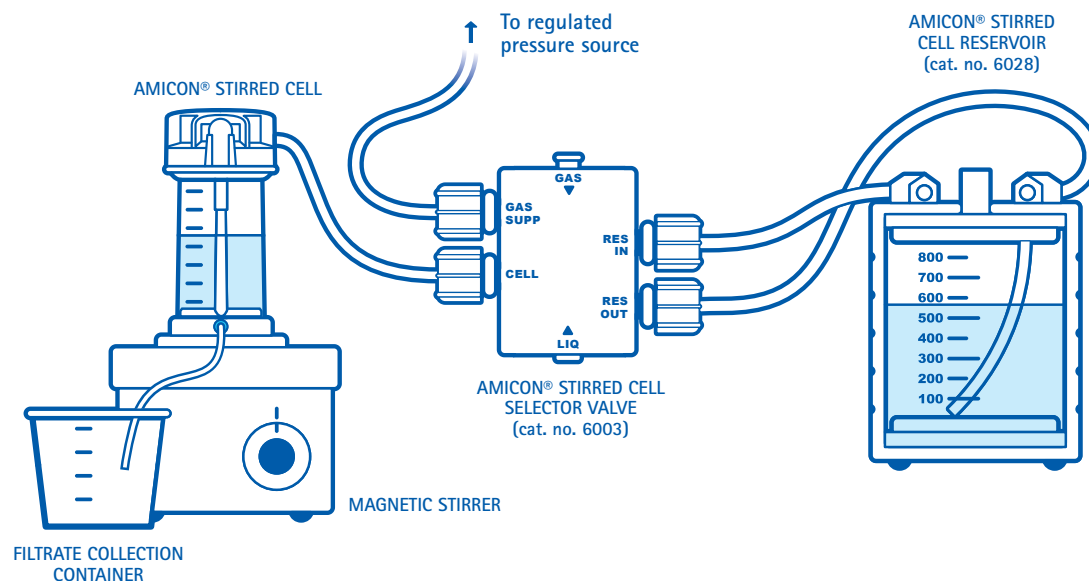


Figure 3. Amicon® Stirred Cell setup for large volume concentration and continuous diafiltration using selector valve and reservoir accessories.

Continuous diafiltration

The concentrated sample (now at 1 mg/mL BSA containing 1 M NaCl) was then buffer-exchanged to remove the sodium chloride, using the previously described stirred cell accessories.

The reservoir was disassembled using the cap removal tool (included in the reservoir kit), cleaned with mild detergent and rinsed with deionized water prior to refilling with 10 mM Tris HCl for salt removal. All fluid-carrying tubing was washed with mild detergent and rinsed with deionized water. The reservoir was again connected to the stirred cell containing the concentrated BSA (1 mg/mL with 1M NaCl) via the selector valve (Figure 3). The conductivity of the starting material was measured prior to desalting to monitor the progress of diafiltration. To begin desalting via continuous diafiltration, the selector valve was set to "Gas" mode (gas spool in) and stirring was initiated on the magnetic stirrer at 200 rpm. Then, pressure was applied at 50 psi, pressurizing both the Amicon® Stirred Cell and the reservoir. After 5-10 seconds, when the pressure was equalized in both stirred cell and the reservoir, the selector valve was shifted to "Liquid" mode (liquid spool in), allowing the liquid in the reservoir to flow into

the stirred cell. The liquid level in the stirred cell was maintained at about 50 mL during the desalting process. The protein concentration and salt conductivity of the concentrate was measured throughout the process to calculate salt reduction over time. The filtration process was stopped once the salt was reduced by 99% (Table 3).

Discontinuous diafiltration

As previously described, 0.1 mg/mL BSA solution containing 1M NaCl was concentrated to 1 mg/mL BSA using large volume concentration. The concentrated sample (now at 1 mg/mL BSA containing 1 M NaCl), was then buffer-exchanged to remove the sodium chloride by discontinuous diafiltration using the Amicon® Stirred Cell. To start the discontinuous buffer exchange, the Amicon® Stirred Cell was disconnected from the selector valve and stirred cell pressure inlet tubing was directly connected to a pressure-regulated nitrogen source.

To initialize discontinuous diafiltration, 50 mL of 10 mM Tris HCl was added to the 50 mL of concentrated BSA solution in the stirred cell. The cap was reinstalled, the slide lock engaged and stirring was initiated prior to application of 50 psi pressure. Concentration continued until 50 mL of permeate was collected, and pressure turned off at the source prior to removal of the cap.

Table 3. Diafiltration volumes required to reduce salt concentration for discontinuous and continuous diafiltration.

Discontinuous Diafiltration				Continuous Diafiltration		
Diafiltration Volumes	Conductivity (μS/cm)	Salt Conc (mM)	% Reduction	Conductivity (μS/cm)	Salt Conc (mM)	% Reduction
Start	84600	1002.9	0	84600	1002.9	0
D1	45100	528.31	47.32	32500	376.92	62.42
D2	23900	273.59	72.72	13430	147.79	85.26
D3	12710	139.14	86.13	5530	52.87	94.73
D4	6860	68.85	93.13	2480	17.72	98.23
D5	3860	30.5	96.96	1311	6.89	99.31
D6	2250	15.59	98.45			
D7	1411	7.82	99.22			

Comparison of Continuous and Discontinuous Diafiltration Removal of Sodium Chloride

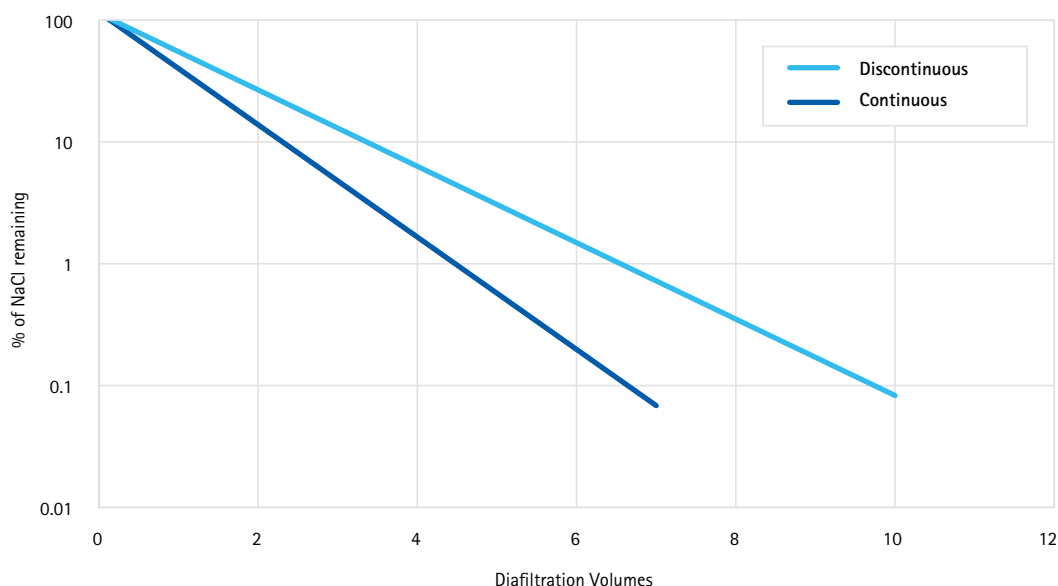


Figure 4. Fewer DVs are required to remove sodium chloride using continuous diafiltration compared to discontinuous diafiltration. Data from Table 3 were plotted to show that continuous diafiltration is more efficient than the discontinuous method.

Protein concentration as well as conductivity measurements were performed on the retentate. To continue, the retentate was once again diluted with 50 mL of 10 mM Tris HCl and the process of concentration and dilution was continued until the salt concentration was reduced by 99%. Table 3 shows conductivity measurements, corresponding salt concentration and % salt reduction after each diafiltration volume. This data is plotted on Figure 4.

Conclusion

Our results show that continuous diafiltration using the Amicon® Stirred Cells enables more efficient buffer exchange with less diafiltration volumes to reach 99% salt reduction compared to discontinuous diafiltration. Furthermore, in continuous mode, the stirred cell does not have to be disassembled between each diafiltration volume.

During continuous diafiltration, the protein concentration stayed constant at 1 mg/mL throughout

the process, while in discontinuous diafiltration, the protein concentration fluctuated significantly between 1 mg/mL and 0.5 mg/mL. Therefore, continuous diafiltration is much more gentler than discontinuous diafiltration, as it maintains product stability by keeping the sample concentration and volume constant during diafiltration.

The flexible, easy-to-use Amicon® Stirred Cells are compatible with a broad range of process volumes (up to 400 mL) that can be further expanded with an addition of an external reservoir, which can be used for large volume concentration as well as batch and constant-volume diafiltration. The new design of the Amicon® Stirred Cells accommodates a wide range of ultrafiltration and microfiltration disc membranes, which can be used to optimize concentration and diafiltration conditions. Unlike centrifugal devices, the pressure-based format provides a gentler method for concentration, reducing the likelihood of shear stress-induced denaturation. Further, the inclusion of magnetic stirring at the filtration interface greatly minimizes the risk of concentration polarization and fouling.

Ordering Information

Amicon® Stirred Cell		
Volume	Membrane Diameter	Catalog No.
50 mL	44.5 mm	UFSC05001
200 mL	63.5 mm	UFSC20001
400 mL	76 mm	UFSC40001

Ultrafiltration Membranes (qty 10/pack)

Material	NMWL (K)	Diameter (mm)*		
		44.5 mm	63.5 mm	76 mm
Ultracel® Membrane	1	PLAC04310	PLAC06210	PLAC07610
	3	PLBC04310	PLBC06210	PLBC07610
	5	PLCC04310	PLCC06210	PLCC07610
	10	PLGC04310	PLGC06210	PLGC07610
	30	PLTK04310	PLTK06210	PLTK07610
	100	PLHK04310	PLHK06210	PLHK07610
Biomax® Membrane	5	PBCC04310	PBCC06210	PBCC07610
	10	PBGC04310	PBGC06210	PBGC07610
	30	PBTK04310	PBTK06210	PBTK07610
	50	PBQK04310	PBQK06210	PBQK07610
	100	PBHK04310	PBHK06210	PBHK07610
	300	PBMK04310	PBMK06210	PBMK07610
	500	PBVK04310	PBVK06210	PBVK07610

*Other diameters available. Please visit www.emdmillipore.com/ufdiscs

Amicon® Stirred Cell Accessories

Item	Description	Catalog No.
Amicon® Stirred Cell Selector Valve	For instant switching from concentration to diafiltration	6003
Amicon® Stirred Cell Manifold	For operating multiple stirred cells in parallel	6015
Amicon® Stirred Cell Reservoir	To increase volume capacity of the stirred cell and for use with diafiltration applications	6028

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