Centrifugal Ultrafiltration Devices for……

protein / nucleic acid concentration, desalting, buffer exchange *and* **more…..**

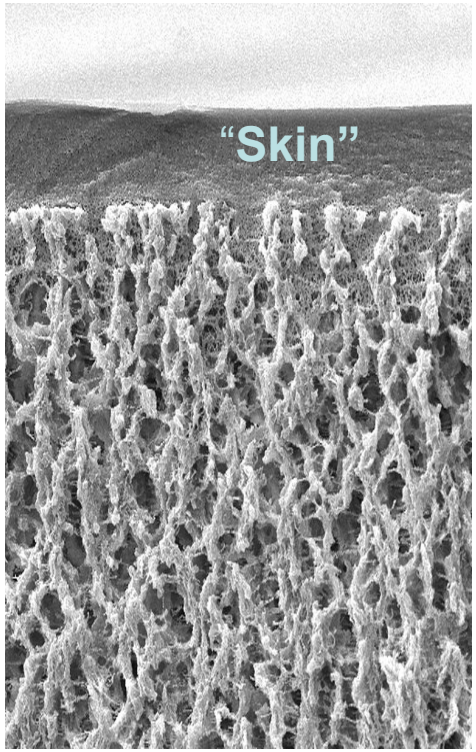
What is Ultrafiltration?

A process for the **separation** and/or **concentration of dissolved macromolecules** using an **ultra-filtration membrane** with a defined cut off

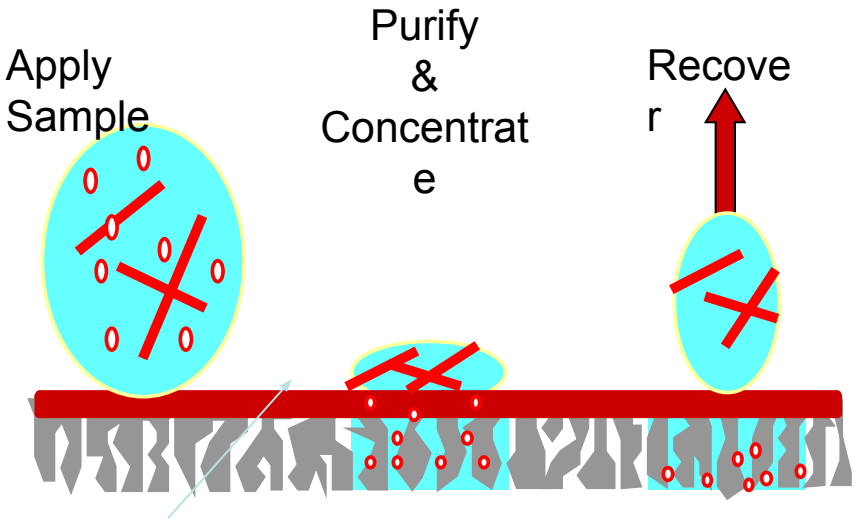
- Primary basis for separation is **molecular weight/size**
- **Driving force** can be **Centrifugal**, **Vacuum** or **Pressure**

What is an Ultrafiltration Membrane?

Structure of Ultrafiltration Membrane



Porous support



The UF 'skin' drives the separation

Ultracel® YM & PL regenerated cellulose

- Dense top layer = ultrafiltration membrane,
- Highly retentive
- Lowest protein binding
- Recommended for proteins; ideal for dilute proteins

How do I select the most suitable device?

- The **molecular weight** and or composition of the sample **determines** NMWL (MWCO) **cut off of the membrane**
- The **volume** and required concentration level of the sample determines **device** selection.
- The **selected device** determines the type of **rotor** and **G force.**

How do I select the most suitable membrane (cut-off) for my protein?

"Molecular Weight of Protein" divided by 2 or 3 = best cut-off of the ultrafiltration membrane

Example:

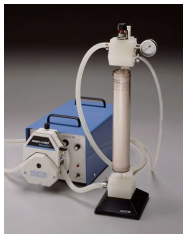
For a **35 kDa** protein, the two potential membrane choices are **10 kDa** or **30 kDa** NMWL.

In this case, only the 10 kDa membrane will provide optimal recovery.

Our current product portfolio for the volume range of 0.5ml up to >10L

Pellicon XL

300 mL - 2 L



Microcon | Amicon Ultra 4 | Amicon Ultra 15 | Centricon Plus-70 | Prep/Scale | Pellicon 2

<1 mL | 1 – 4 mL | 4 – 30 mL | 30 - 420 mL | 2 – 10 L | >10 L

Stirred Cells

100 - 2000 mL

How to select the best device by volume?

Maximum Starting volume	**< 1 mL**	**1 to 8 mL**	**4 – 30 mL**	**30 - 420mL**
Final volume	**5 - 15µL**	**50µL**	**200µL**	**350µL**

Microcon … the lab standard for small volumes

High recovery design

- Regenerated cellulose membrane
  - low binding
  - highly retentive
  - High recovery
- Invert spin concentrate recovery
- Low binding materials and o-ring seal
- Final conc. Volume 5 - 15 µL
- cut offs 3K, 10K, 30K, 50K, 100K

Microcon in DNA / RNA applications – Use the Selection Guideline in the protocole

Centrifugation Guidelines for Microcon Devices

Ultracel YM Membrane	Color Code	Membrane NMWL	NCO SS	NCO DS	Maximum g-Force	Spin Times* 25 °C**
YM-3	Yellow	3,000	10	10	14,000	100
YM-10	Green	10,000	30	20	14,000	30
YM-30	Clear	30,000	60	50	14,000	12
YM-50	Rose	50,000	125	100	14,000	12
YM-100	Blue	100,000	300	125	14,000	12
					500[1]	12

NMWL: Nominal molecular weight limit in Daltons (proteins)
NCO: Nucleotide cut-off for RNA/DNA (SS = single stranded, DS = double stranded)
*Time in minutes; 500 µL samples reduced to 10 µL.
**Sample concentration using Microcon devices at 4 °C typically takes twice as long as that at 25 °C.
[1]For RNA/DNA samples using Microcon devices with Ultracel YM-100 membrane, the recommended g-force is 500 × g.
NOTE: DNA sample concentration using Microcon devices generally requires shorter spin times. For example, at 25 °C, a 10 µL final volume of an RNA/DNA sample can be achieved in a Microcon device with Ultracel YM-30 membrane in just 8 minutes.

The next stage in centrifugal filter devices.

Amicon® Ultra

1 – 4 mL | 4 – 15 mL

Designed for speed and convenience -- without sacrificing recovery

- 5 - 45 minute processing times
- Single-spin operation -- recover concentrate by pipette
- Universal rotor compatibility

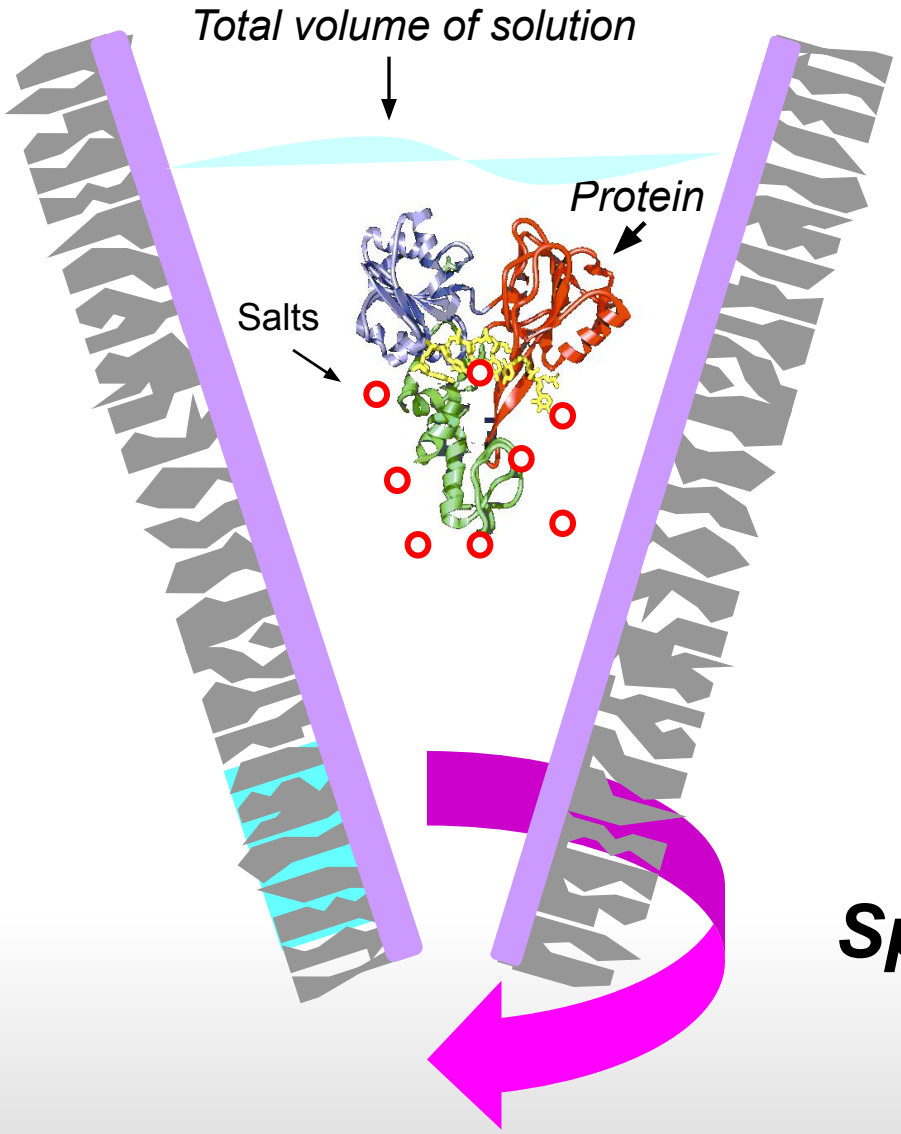
Low-binding Ultracel ultrafiltration membrane

- Retentate recoveries >90%
- Broad chemical compatability

Selecting a Centrifugal Device

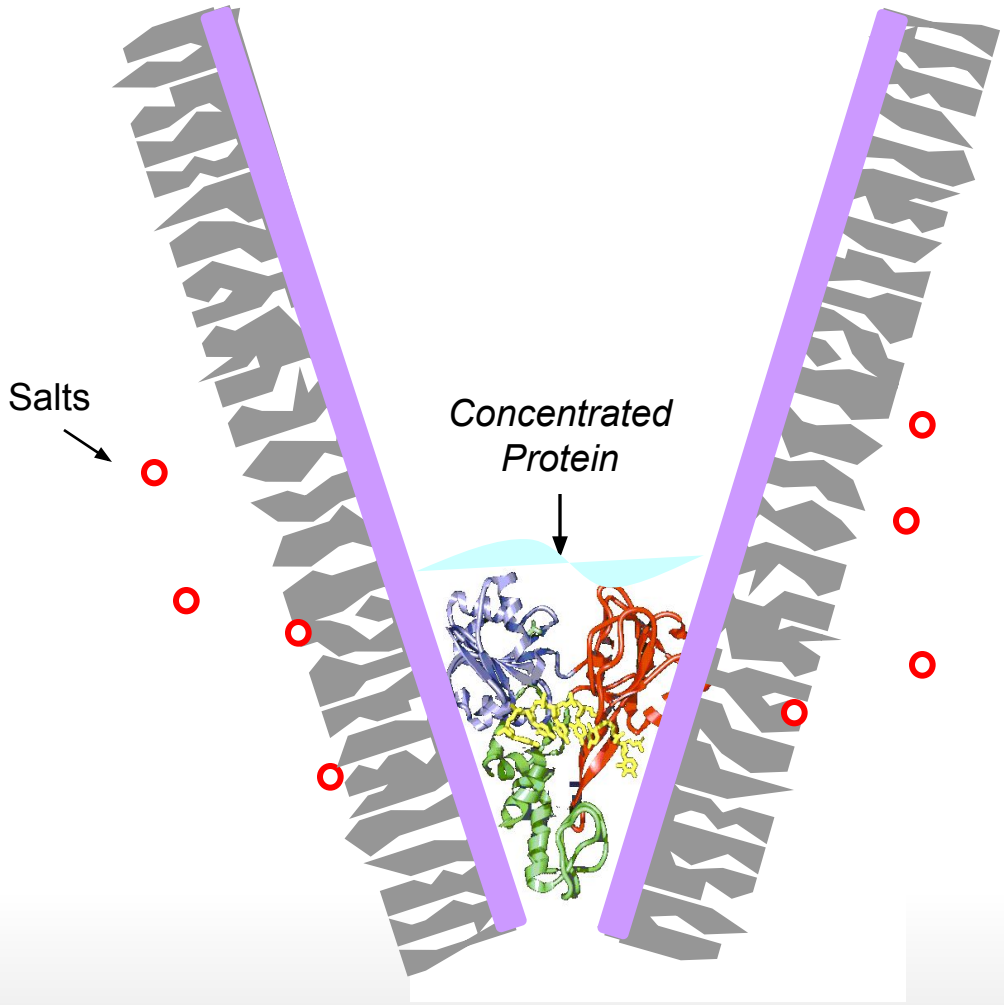
- A vertical membrane design applies the principles of TFF and accelerates concentration times
- Throughput is important when working with larger volumes.
- A dead-stop can prevent spinning to dryness

Amicon Ultra
Mode of Action



Spin….

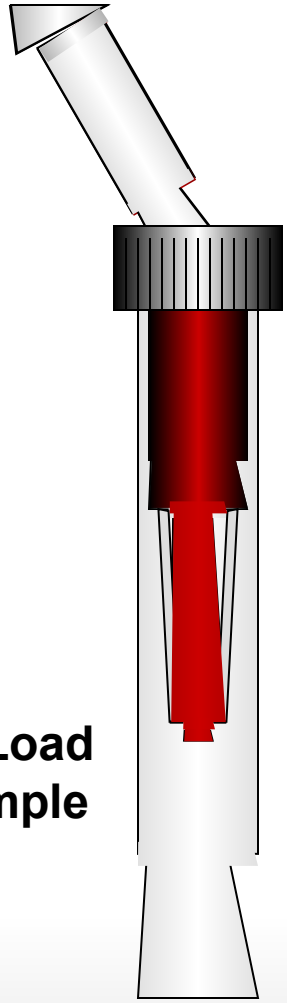
Amicon Ultra
Mode of Action



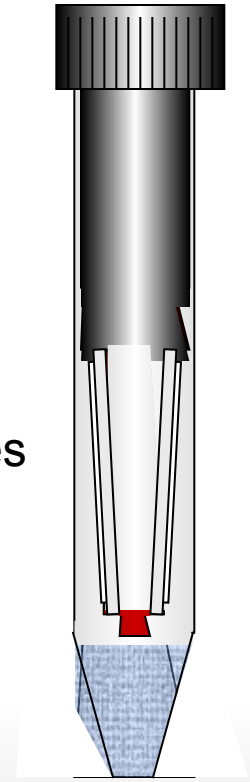
1 – 4 mL | 4 – 15 mL

Fast and Easy

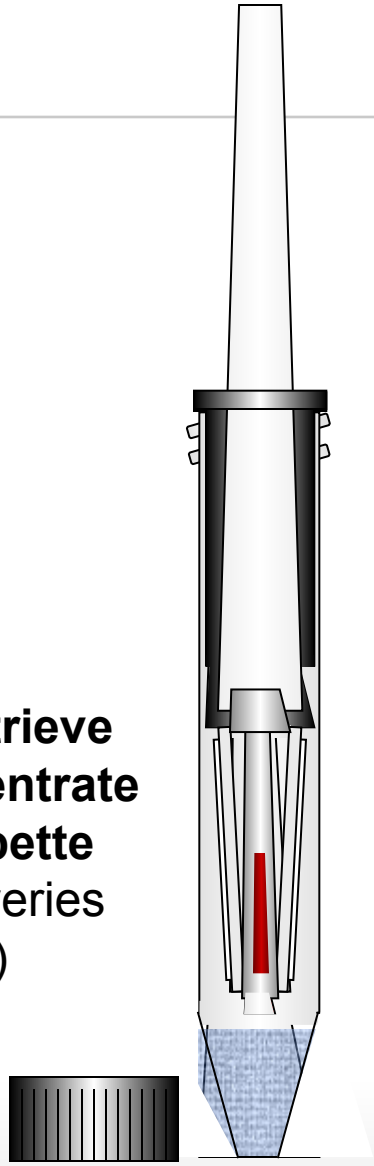
Single-spin operation



1) **Load sample**



2) **Spin** (typical spin times 10-20 minutes)



3) **Retrieve concentrate by pipette** (recoveries >90%)

Select the correct device size:

Samples 2 - 8 mL = 4 mL device
Cut offs: 5K, 10K, 30K, 100K

Samples 10 - 30 mL = 15 mL device
Cut offs: 5K, 10K, 30K, 100K

Amicon Ultra - Product Offering

Model & Volume	Membrane Type	MWCO	8/pk	24/pk	96/pk
Amicon Ultra-4 4 ml	Ultrace low-binding regenerated cellulose	**3 kD**	UFC800304	UFC800324	UFC800396
		10 kD	UFC801008	UFC801024	UFC801096
		30 kD	UFC803008	UFC803024	UFC803096
		50 kD	UFC805008	UFC805024	UFC805096
		100 kD	UFC810008	UFC810024	UFC810096
Amicon Ultra-15 15 ml	Ultrace low-binding regenerated cellulose	**3 kD**	UFC900308	UFC900324	UFC900396
		10 kD	UFC901008	UFC901024	UFC901096
		30 kD	UFC903008	UFC903024	UFC903096
		50 kD	UFC905008	UFC905024	UFC905096
		100 kD	UFC910008	UFC910024	UFC910096

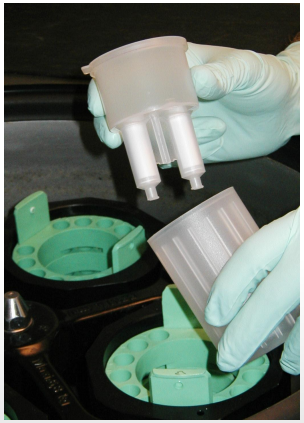
Centricon Plus-70
Large Volume Device

30 - 70 mL

Concentrates down to 350 µL

in 15 to 20 minutes.

- Efficiency with double core vertical membrane
- Dead stop with invert spin
- Low-binding Ultracel ultrafiltration membrane



MILLIPORE

How to select the type of rotor and G force

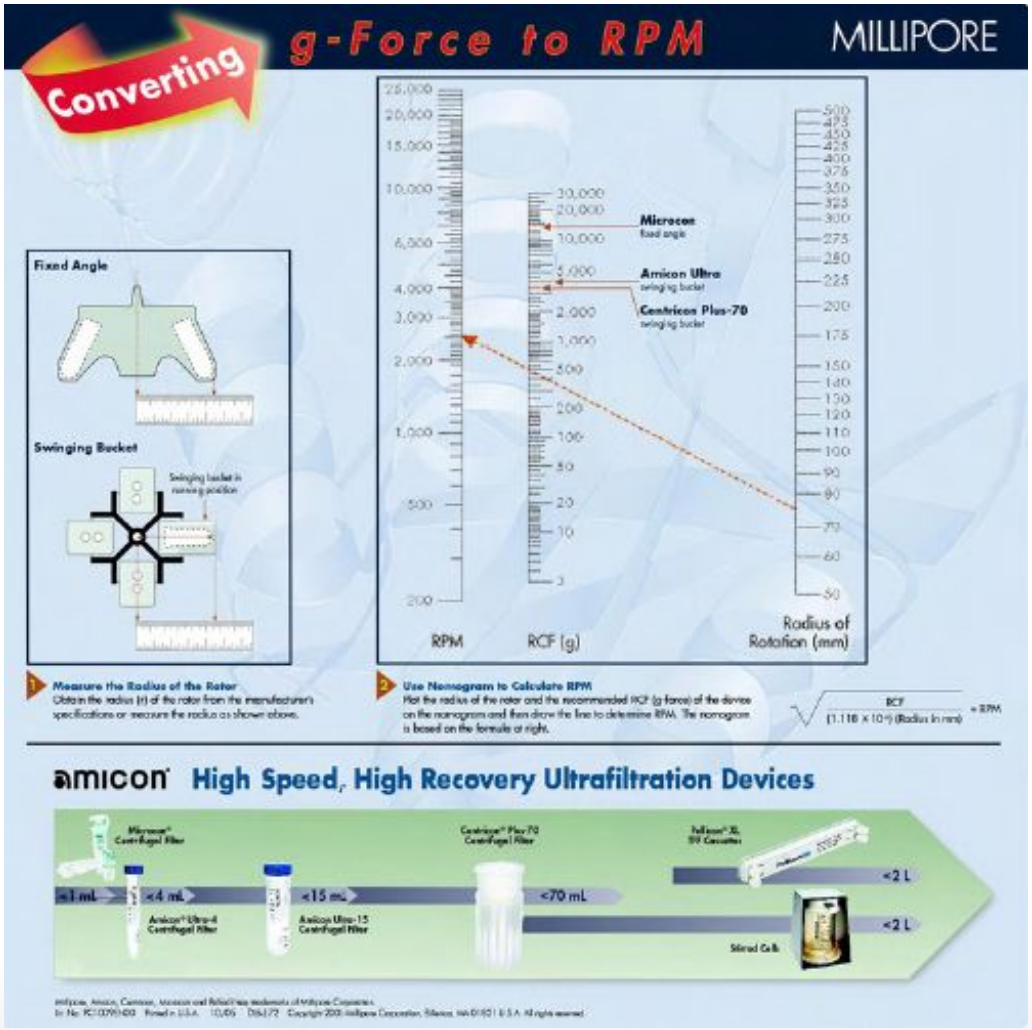
RPM

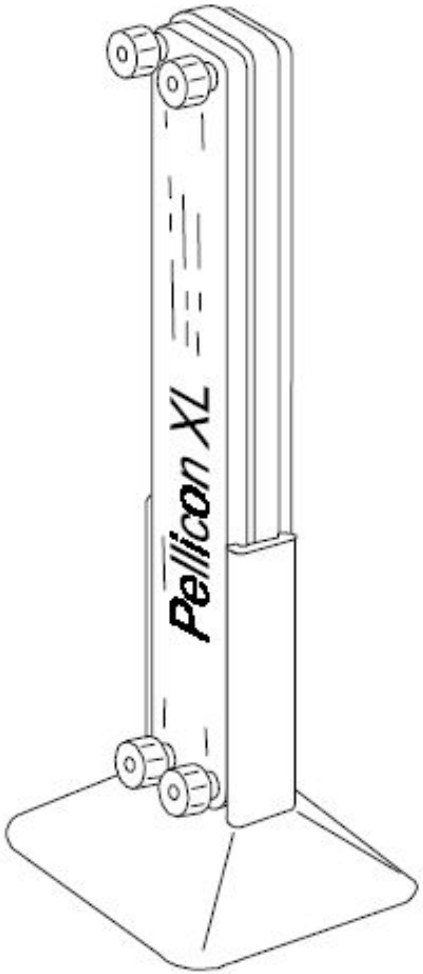
↑

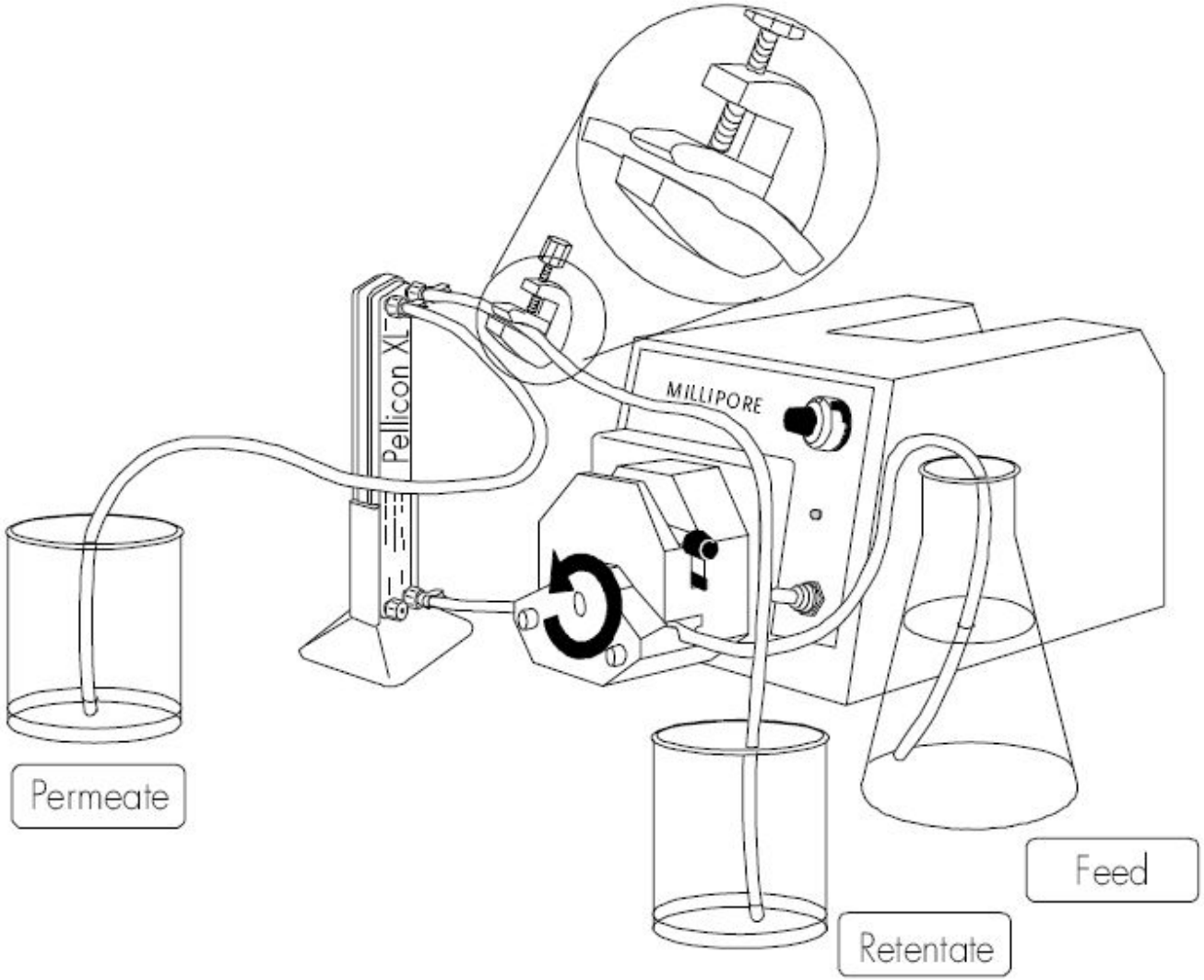
RCF (g) max with each device

↑

Radius of rotation (mm)




Pellicon XL


Pellicon XL
MILLIPORE
Permeate
Feed
Retentate

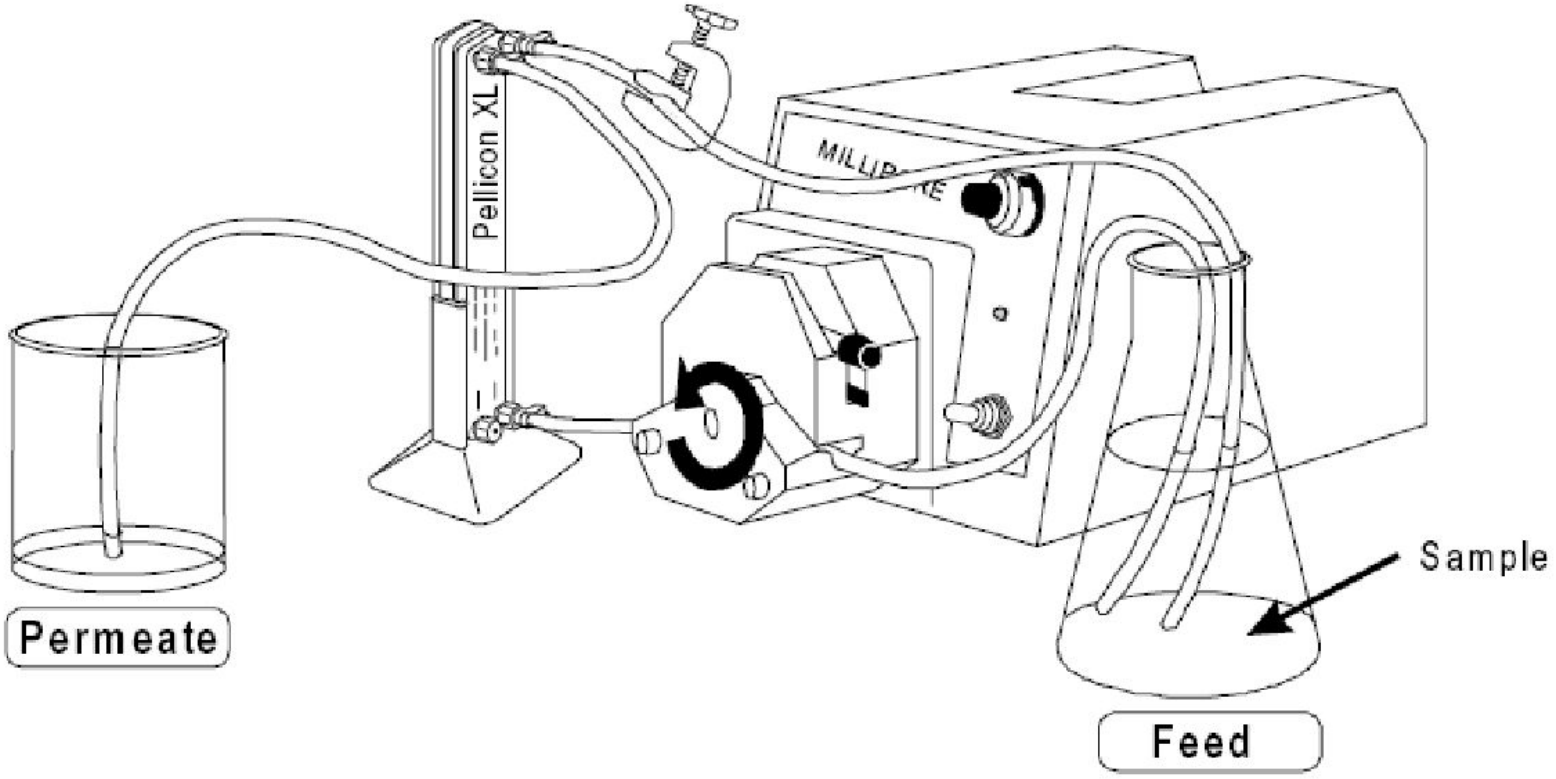


Figure 5: Sample Processing

Section VII: Cleaning

Recommended cleaning solutions (marked by X):

Cleaning Agents	Concentration	Catalogue Numbers with prefix PXB Biomax™ Membrane (polyethersulphone)	Catalogue Numbers with prefix PXC Ultracel™ PLC Membrane (composite regenerated cellulose)	Catalogue Numbers with prefix PXV, PXG, PXH, PXD Durapore® Membrane (PVDF)	Temp ° C	pH	Time (min)
NaOH	0.1-0.5N	X			40-45	13-13.7	30-60
NaOH	0.1N		X		25-40	13	30-60
NaOCl	250 ppm	X		X	40-45	10-11	30-60
Triton®-X 100	0.10%	X	X	X	40-45	5-8	30-60
SDS	0.10%	X	X	X	40-45	5-8	30-60
Tween® 80	0.10%	X	X	X	40-45	5-8	30-60
Terg-a-zyme® Detergent	0.20%	X	X	X	40-45	9-10	30-60

Do not exceed 30 psi inlet for cleaning cycle.

Section VIII: Storage

Recommended storage solutions:

Storage solution	Concentration	Catalogue Numbers with prefix PXB Biomax Membrane (polyether-sulphone)	Catalogue Numbers with prefix PXC Ultracel PLC Membrane (composite regenerated cellulose)	Catalogue Numbers with prefix PXV, PXG, PXH, PXD Durapore Membrane (PVDF)
NaOH	0.1N	X		
NaOH	0.05N		X	
Lysol® (BAK)	0.1%	X	X	X
Sodium Azide	0.05%	X	X	X
H_3PO_4	0.1N	X	X	X

Centrifugal devices and more….

Application Information available

Amicon Bioseparations Information Topics

- Detergent removal
- Passivation (reduce protein binding)
- Desalting and buffer exchange
- Depyrogenation
- Protein, DNA, RNA purification
- Protein and DNA concentration
- PCR product purification
- Extraction DNA from gels
- Peptide Concentration
- UF Selection guide

Thank for your attention