

# Sartobind Membrane Adsorber Units

## Product Specification

### A New Separation Technology Based on Microporous Membrane Ion Exchangers

Ready-to-use Sartobind membrane adsorber units are the ultimate for simple, ultra-rapid concentration of proteins, peptides, nucleotides, plasmids, DNA fragments, etc. from highly dilute solutions, or for separating proteins from a mixture. They feature high binding capacity (~1mg/cm<sup>2</sup>), high flow rates (up to 100+ mL/min), and excellent resolution. Use these units with syringes, peristaltic pumps, or preparative HPLC/FPLC® workstations – you will not lose time in changing from a traditional chromatography column to an adsorber unit. Procedures which formerly required an hour or more will be accomplished in minutes.

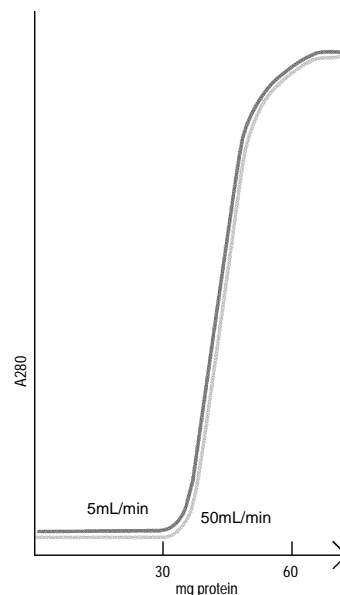
Four types of membrane ion exchangers, S (sulfonic acid), Q (quaternary ammonium), C (carboxyl groups) and diethylamine (D) are available (Table 1), in 5cm<sup>2</sup> disposable units (MA5 units) and 15cm<sup>2</sup> and 100cm<sup>2</sup> reusable units (MA15 and MA100 units).

**Table 1. Functional Groups on Sartobind Membranes**

Group (Membrane Type)/Exchange Type	Structure
Sulfonic acid (S)/strongly acidic cation exchanger	R-CH <sub>2</sub> -SO <sub>3</sub> <sup>-</sup>
Quaternary ammonium (Q)/strongly basic anion exchanger	R-CH <sub>2</sub> -N <sup>+</sup> -(CH <sub>3</sub> ) <sub>3</sub>
Carboxyl groups (C)/weakly acidic cation exchanger	R-COO <sup>-</sup>
Diethylamine (D)/weakly basic anion exchanger	R-CH <sub>2</sub> -N <sup>+</sup> -(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>

**Figure A. Breakthrough Curves for Cytochrome c on an S15 Unit**




Separation performance is virtually independent of flow rate



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**Table 2. Specifications for Sartobind Membrane Adsorber Units**

All units pass USP cytotoxicity test with MRC-5 human embryonic lung cells; USP Plastics Class VI test.

Specification	MA5 Units Disposable	MA15 Units Reusable	MA100 Units Reusable
			
Adsorption Area*	5cm <sup>2</sup>	15cm <sup>2</sup>	100cm <sup>2</sup>
Membrane Material	-----	crosslinked regenerated cellulose	-----
Housing Material	MBS-copolymer	polysulfone	polysulfone
Inlet/Outlet Connector	-----	female Luer lock/male Luer lock	-----
Hold-Up Volume	0.8mL	1.0mL	4.2mL
Protein Binding Capacity**	>5mg/S5 unit >3mg/Q5 unit >3mg/C5 unit >2mg/D5 unit	>15mg/S15 unit >9mg/Q15 unit >9mg/C15 unit >6mg/D15 unit	>100mg/S100 unit >60mg/Q100 unit >60mg/C100 unit >40mg/D100 unit
Recovery with 1M KCl	>90%	>90%	>90%
Flow Rate at 14psi	>150mL/min	>50mL/min	>75mL/min
Maximum Pressure	60psi	105psi	90psi
pH Stability	2-13	2-13	2-13
Storage Before Use	-----	dry at room temperature	-----
Regeneration	—	1N NaOH or 1N HCl, 1 hr, room temperature. Backflushing is possible.	—
Storage After Use	—	Wet (buffer or saline solution + antimicrobial agent), 4-8°C. Do not expose to pure water or to organic solvents.	—

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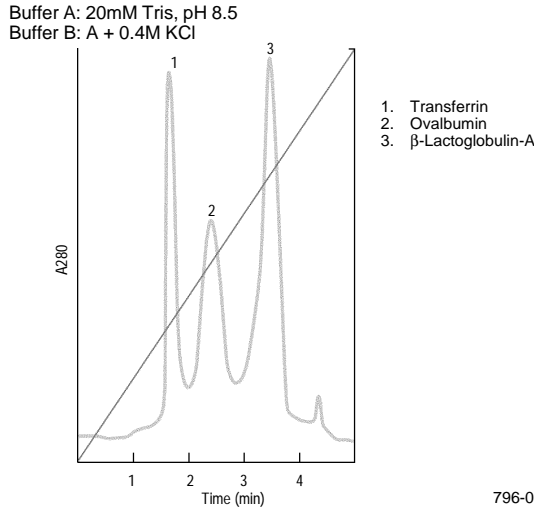
\* 50cm<sup>2</sup> adsorption area ≅ 1mL membrane volume.

\*\* Depends on adsorber type and conditions used. Reference proteins: lysozyme for cation exchangers, bovine serum albumin for anion exchangers.

## Typical Time Savings and High Performance in Laboratory Separations

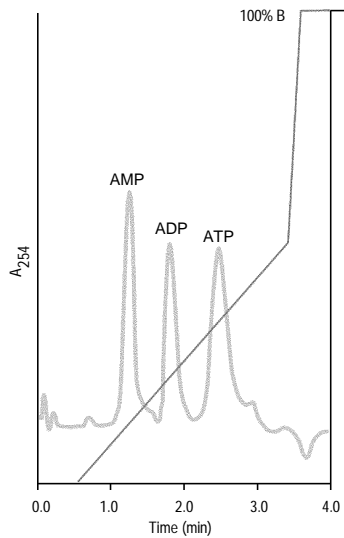
### Figure B. Protein Standards Sharply Separated at a High Flow Rate: Q15 Unit / 50mL/min

Separations are excellent at flow rates up to 100mL/min



### Figure C. Excellent Separation of Nucleosides: D15 Unit / 8mL/min

Buffer A: 25mM Tris, pH 7.5  
Buffer B: A + 1M NaCl

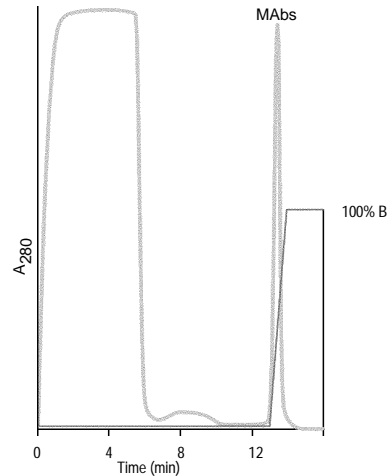


## Purification of Monoclonal Antibodies (MAbs)

Sartobind Membrane Adsorber Units effectively and rapidly purify MAbs, especially from supernatants of serum-free cell cultures. They reduce albumin, transferrin, insulin, and other contaminants by more than 90%, through rapid adsorption/desorption or in an FPLC system (Figure D).

### Figure D. MAbs: S15 Unit / FPLC System

Buffer A: 25mM MES (2-[N-morpholino]ethane sulfonic acid), 10mM NaCl, pH 5.8  
Buffer B: 25mM MES, 250mM NaCl, pH 5.8  
Flow Rate: 10mL/min  
Sample: preliminary filtered supernatant of serum-free tissue culture (9.4mg MAbs)

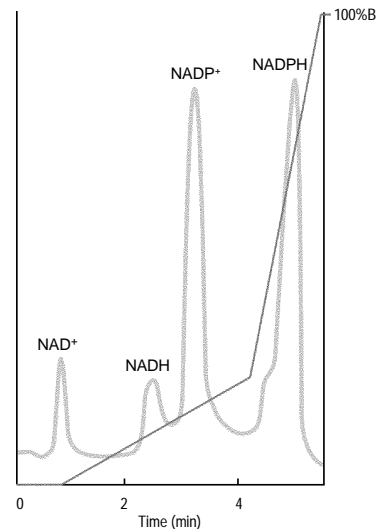


## Rapid Separation of Nucleotides

The adsorber units rapidly separate small molecular weight charged substances. Figure E shows a 5 minute separation of nucleotides.

### Figure E. Nucleotides: Q15 Unit / FPLC System

Buffer A: 0.02M TEA (triethylamine), pH 7.7  
Buffer B: A + 1M KCl, pH 7.7  
Flow Rate: 1.0mL/min  
Sample: 50 $\mu$ M NAD<sup>+</sup>, NADH, 150 $\mu$ M NADP<sup>+</sup>, NADPH

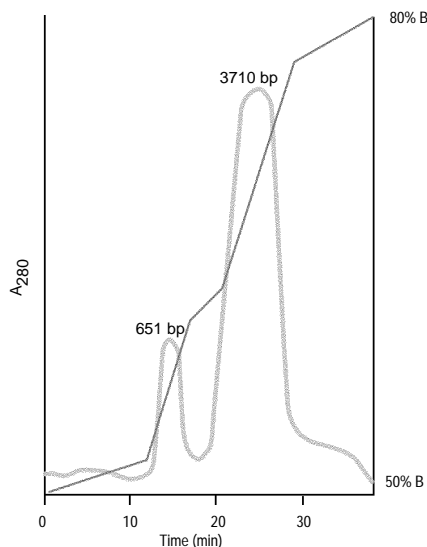


## Microfractionation of DNA Fragments

Sartobind Membrane Adsorber Units are ideal for separating DNA fragments of differing sizes from plasmids or other vectors (Figure F).

**Figure F. DNA Fragments: Q15 Unit / FPLC System**

Buffer A: 10mM Tris-HCl, 1mM EDTA (ethylenediaminetetraacetic acid), 0.5M NaCl, pH 8.0  
 Buffer B: A + 0.8M NaCl, pH 8.0  
 Flow Rate: 0.1mL/min  
 Sample: 25µL (1.25µg) Eco RI/Sal digested pBR322 DNA



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## Rapid Concentration and Purification of Proteins, Using Membrane Adsorber Units in an Adsorption/Desorption Mode

The disposable adsorber units (5cm<sup>2</sup> adsorption area) are ideal for concentrating and purifying proteins from small volumes of sample. Additionally, these units are optimal for removing contaminants and for screening tests. The larger, reusable units (15cm<sup>2</sup> and 100cm<sup>2</sup> adsorption areas) are intended for larger volumes, up to 20 liters, and higher protein quantities (Table 2). High dynamic binding capacity, accommodation of flow rates up to 200+ mL/min, and easy handling allow the use of a syringe or peristaltic pump for rapid binding (adsorption) and elution (desorption) in concentration and purification procedures.

## Rapid Protein Concentration

Flow across the adsorber units increases linearly with pressure, thus separation and/or concentration can be performed rapidly. Very dilute pure or partially pure proteins can be concentrated easily by pressure filtration (Table 3).

**Table 3. Protein Concentration, Using Reusable Adsorber Units**

Unit	S15	Q15	
Protein	cytochrome c	bovine serum albumin	
Volume	200mL	200mL	
Loading Buffer	10mM Na phosphate, pH 7.0		
Washing Buffer	10mL loading buffer		
Elution	1M KCl, 3mL		
<b>Results:</b>	<b>Initial</b>	<b>Final</b>	<b>Evaluation</b>
Volume	200mL	3mL	98.5% reduction
Concentration	0.05mg/mL	6.75mg/mL	66-fold conc.
Protein Recovery			95-100%
Time			5-7 minutes

Samples processed manually via syringe.

## Protein Purification

Liter volumes of protein solutions can be processed by using a 100cm<sup>2</sup> adsorber unit connected to a suitable peristaltic pump (Figure G). The protein is purified in the adsorption/desorption mode – binding and elution can be accomplished at flow rates of 100-200mL/min. For example, an S100 unit and a flow rate of 100mL/min were used to concentrate 3 liters of solution containing 0.04mg mouse/mouse IgG<sub>1</sub>/mL to 10mL, with 100% recovery of the protein. The 300-fold concentration was completed in less than 40 minutes.

**Figure G. Sartobind Membrane Adsorber Unit Connected to a Peristaltic Pump**



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## Removal of Contaminants

DNA fragments, endotoxins, viruses, cell culture components, and other contaminants can be separated easily and rapidly from the target substance. Selection of the appropriate conditions will bind the target substance and allow the contaminants to pass, or will bind the contaminants and allow the target substance to pass. Tables 4, 5 and 6 show typical capabilities of these units.

**Table 4. Rapid Purification of Monoclonal Antibodies with an S15 Unit**

Protein	mouse/mouse IgG <sub>1</sub>
Initial Volume	200mL
Initial Concentration	0.02mg/mL
Contaminants	BSA (1mg/mL), human transferrin, bovine insulin (0.01mg/mL each)
Flow Rate	50mL/min (via syringe)
Loading Buffer (A)	25mM MES + 10mM NaCl, pH 5.9
Washing Buffer	20mL A, then 10mL A + 25mM NaCl
Elution	5mL A + 250mM KCl

### Results:

	Initial	Final	Evaluation
Volume	200mL	5mL	97.5% reduction
Concentration	0.02mg/mL	0.72mg/mL	36-fold conc.
Purity	–	>90%	SDS-PAGE + ELISA
Protein Recovery	4mg	3.6mg	90%
Time			6-7 minutes

In Table 5, levels of stock endotoxin from *E. coli* or endotoxin native to the sample were quantified through an LAL test. Removal of endotoxin with a Sartobind Membrane Adsorber Unit at high flow rates provides process development scientists with a new tool for purifying pharmaceuticals, and enables tissue culturists to protect sensitive cell cultures.

**Table 5. Log Reduction of Endotoxins with a Q100 Unit**

Solution (EU/mL)	Initial	Final	Log Reduction
Water	10,000	<0.06	>5.22
Buffer (PBS)	10,000	0.96	4
BSA	6	<0.06	2
γ-Globulin	101	<0.06	>3.2
Monoclonal Antibody	100	<0.06	>3.2
Protein from <i>E. coli</i> culture (3 passes)	~5 x 10 <sup>6</sup>	50	5

Table 6 shows monoclonal antibodies from clarified cell culture were purified to 95% in two ion exchange steps, first with an S100 unit (antibodies trapped, viruses passed), then, after antibody elution and buffer adjustment, with a Q15 unit (viruses trapped, antibodies passed). Viruses were assayed before and after each step, to demonstrate clearance of viruses during purification.

**Table 6. Log Reduction of Viral Particles from Monoclonal Antibodies**

	Polio	Herpes	MuLV
S100 Unit	1.52	3.36	2.69
Q15 Unit	1.64	4.81	4.82
Total Removal	3.16	8.17	7.51

## Screening Tests

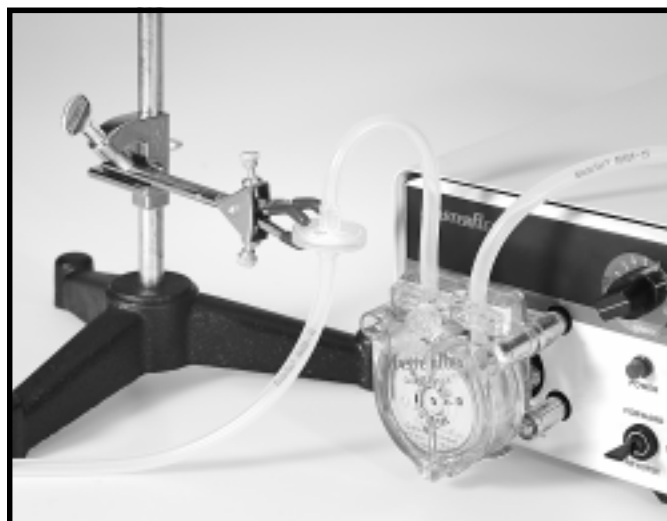
Disposable 5cm<sup>2</sup> adsorber units can be used for establishing the optimal purification and concentration conditions for proteins not yet characterized. The time needed for sample screening is 1/5 to 1/10 that for the fastest particle-based devices available.

## Effects of Flow Rate and Number of Cycles on Resolution and Separation Efficiency

Sartobind Membrane Adsorber Units can be used with the protein separation program in an FPLC system (Figure H). Luer lock inlet/outlet connectors on the adsorber units make installation very easy. Workstations with high performance flow rates (e.g., the BioPilot system) can be used, to take full advantage of the reproducible separations which are possible at high flow rates. Figure I shows the separations of reference proteins at three flow rates on a BioPilot system. There was no loss in separation performance or capacity, even when the cycle time was reduced by a factor of 10.

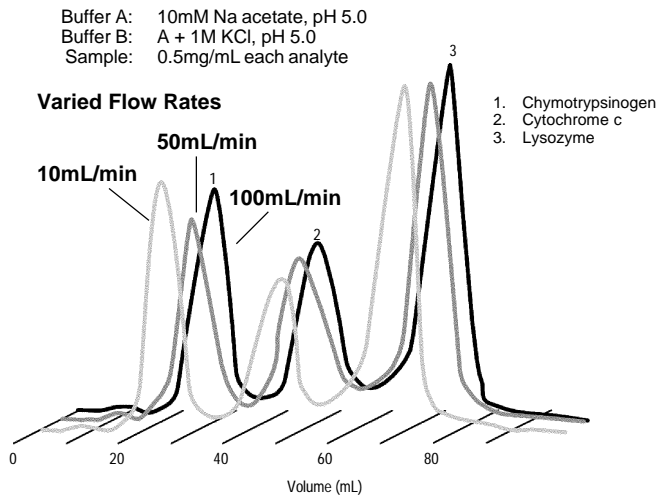
The chemical stability of the adsorbers (pH 2-13) allows regeneration with aggressive cleaning agents, e.g., 1N NaOH for one hour. Figure I shows 100 cycles of protein separation (equilibration - loading - washing - elution with KCl - regeneration with 0.2N NaOH) in an FPLC system did not change resolution.

**Figure H. Adsorber Unit Connected to an FPLC System**

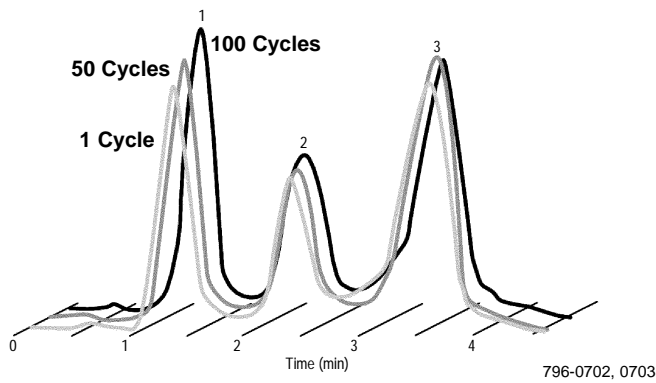


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**Figure I. Consistent Separation of Reference Proteins with an S15 Unit**



**After Many Cycles (20mL/min)**



## Scaleability of Applications on Membrane Adsorbers

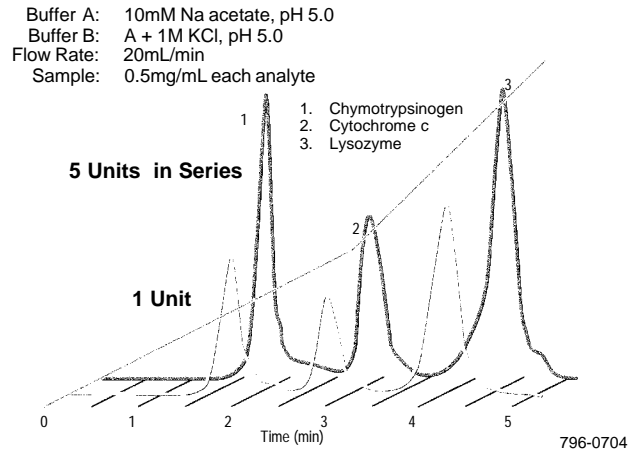
### Connect Adsorber Units in Series

With a workstation, separation capacity can be increased very easily, by connecting several adsorber units in series. Elution volume remains low and, therefore, the concentration of the target substance is increased. Protein separation can be improved (Figure J). A syringe filter can be directly connected to an adsorber unit, to combine preliminary filtration and protein concentration/separation into one step. It is also easy to combine different adsorber types in series. Refer to the Ordering Information on page 6 of this product specification for a list of connectors for workstations.

### Transfer Separations from MA15 Units onto MA100 Units

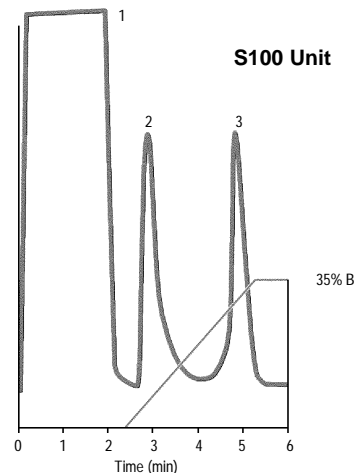
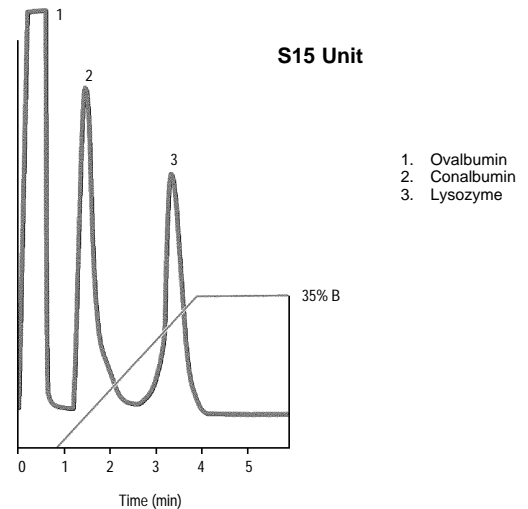
Binding capacity of Sartobind Membrane Adsorber Units increases linearly with adsorption area. This facilitates transferring a procedure from small (development) scale to large (process) scale. An example is shown in Figure K. To isolate egg white proteins at preparative scale, the conditions used with the S15 unit were transferred to the S100 unit. Resolution was similar.

**Figure J. Resolution Improved by Connecting S15 Units in Series**



**Figure K. Linear Increases in Binding Capacity Make Scale-Up Easy**

Buffer A: 20mM Na acetate, pH 5.3  
 Buffer B: A + 1M KCl, pH 5.3  
 Flow Rate: 40mL/min (S15) or 100mL/min (S100)  
 Sample: egg white proteins, 25mg (S15) or 120mg (S100)



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## Ordering Information:

### Sartobind Ion Exchange Membrane Adsorber Units

Membrane Description	Cat. No.
<b>MA5 units: disposable, 5cm<sup>2</sup> surface area, pk. of 15.</b>	
S-type exchanger	S5F
Q-type exchanger	Q5F
CM-type exchanger	C5F
DEAE-type exchanger	D5F
<b>MA15 units: reusable, 15cm<sup>2</sup> surface area, pk. of 2.</b>	
S-type exchanger	S15F
Q-type exchanger	Q15F
CM-type exchanger	C15F
DEAE-type exchanger	D15F
One S and one Q unit	SQ15F
One CM and one DEAE unit	CD15F
<b>MA100 units: reusable, 100cm<sup>2</sup> surface area, pk. of 1.</b>	
S-type exchanger	S100F
Q-type exchanger	Q100F
CM-type exchanger	C100F
DEAE-type exchanger	D100F

All packages include one Minisart 0.2µm syringe filter for preliminary filtration.

### To Connect Membrane Adsorber Units to a Workstation

Upchurch Connector	Supelco Cat. No.
1/4-28 male	55072
1/4-28 female	55075
10/32 male	55073
10/32 female	55076
M6 male	55074
M6 female	55077

For additional information about these products, contact the Supelco Customer Service Department:

#### Telephone

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outside USA or Canada	814-359-3441

#### FAX

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#### Acknowledgment

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## Related Products

### Centribind Centrifugal Membrane Adsorber Units

These ready-to-use units with an integrated membrane adsorber disc offer a very small minimum elution volume: 0.05mL. They require no special storage and no equilibration – simply remove one from the box, pipette up to 0.5mL of sample into the upper chamber, and spin. S-type, Q-type, C-type, and D-type ion exchangers are available, and a unit with an aldehyde group can be used for affinity ligand attachment.

### Centrisart® I Centrifugal Concentrators

These ready-to-use units are for small volume centrifugal ultrafiltration, for preparing protein-free supernatants, or for recovering low molecular weight substances from biological fluids. A unique design prevents premature blockage of the membrane. Minimum/maximum sample size: 0.5mL/2.5mL; final concentrate volume: ~0.1mL. Available with 5kD, 10kD, 20kD, and 100kD molecular weight cutoffs.

### Centrisart C4 Centrifugal Microconcentrators

Centrisart C4 microconcentrators are designed for quick and efficient small volume ultrafiltration or microfiltration. Five types are available. Major applications for the 5kD, 10kD, 20kD, and 100kD units are concentrating and purifying proteins and DNA, buffer exchange, desalting, and removing low molecular weight substances such as primers, non-incorporated nucleotides, and excess linkers. The maximum sample volume is 0.4mL; the final concentrate volume can be as little as 5-10µL.

### Centrisart C30 Centrifugal Concentrators

Centrisart C30 units are a centrifugal filtration unit for rapidly concentrating small sample volumes by ultrafiltration, and for removing particles. They can be used without adapters in all laboratory centrifuges that accept standard 17mm tubes. The sample volume is 3mL, and the final volume can be as little as 30-50µL. 10kD, 20kD, and 100kD units are available.

### Sartoco® Micro Crossflow Ultrafiltration Units

These sterile, pyrogen-free, ready-to-connect units bridge the gap between centrifugal units and crossflow cassette systems. They can be used to concentrate or purify 50mL to 1 liter volumes. The tangential flow across the filter is gentle on the sample, preventing the foaming and denaturation which can occur in stirred cells. Sartoco Micro crossflow ultrafiltration units offer ease of handling and the user safety of closed, sterile units.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website ([www.sigma-aldrich.com](http://www.sigma-aldrich.com)), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

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