



USER GUIDE

Apollo® 20 mL

High-Performance Centrifugal Concentrators

Note: This product is offered for research use only. Not for clinical use, diagnostic procedures, or for preparation of fluids to be used for human injection. This is a pilot production product which is in final development. Preliminary applications data are available, and a Quality Control System is in place for this product.

Apollo 20 mL UF concentrators are disposable ultrafiltration devices for the concentration or purification of protein solutions. They are far superior to alternatives in combined simplicity, speed, capacity and recovery. This is due to their unique conical design (US Patent 6,269,957, US Patent 6,357,601, PCT patents pending), providing a high ratio of membrane area to sample size. This, in turn, provides a high degree of concentration in a single spin as well as better control of polarization and fouling at the membrane surface. Apollo has the largest available sample volumes for a given centrifuge tube size.

SPECIFICATIONS

Volumes

		<u>Maximum Sample</u>
34° angle rotor:		13.5 mL
With swing-head rotor:		20 mL
Total Volume		
<u>In Concentrator & Filtrate Tube</u>		
<u>Swing Head</u>	<u>34° Angle</u>	<u>Resulting Deadstop</u>
NA	<= 14 mL	13µL* / 28 µL**
<= 20.0 mL	20.6 mL	67 µL
20.6 mL	21.3 mL	100 µL
22.0 mL	22.4 mL	200 µL
24.7 mL	24.4 mL	500 µL
27.2 mL	26.2 mL	1 mL
28.9 mL	27.4 mL	1.5 mL
30.2 mL	28.5 mL	2 mL

* With any port oriented outboard

** With any port oriented inboard

Maximum Centrifugal Force

With 35° angle rotor: 13,000 rcf

With swing-head rotor: 4,500 rcf typical rotor maximum, not to exceed 13,000 rcf

Materials

Membrane: Regenerated cellulose on polyethylene microporous support
 Concentrator, collection tube and cap: Polypropylene

Dimensions

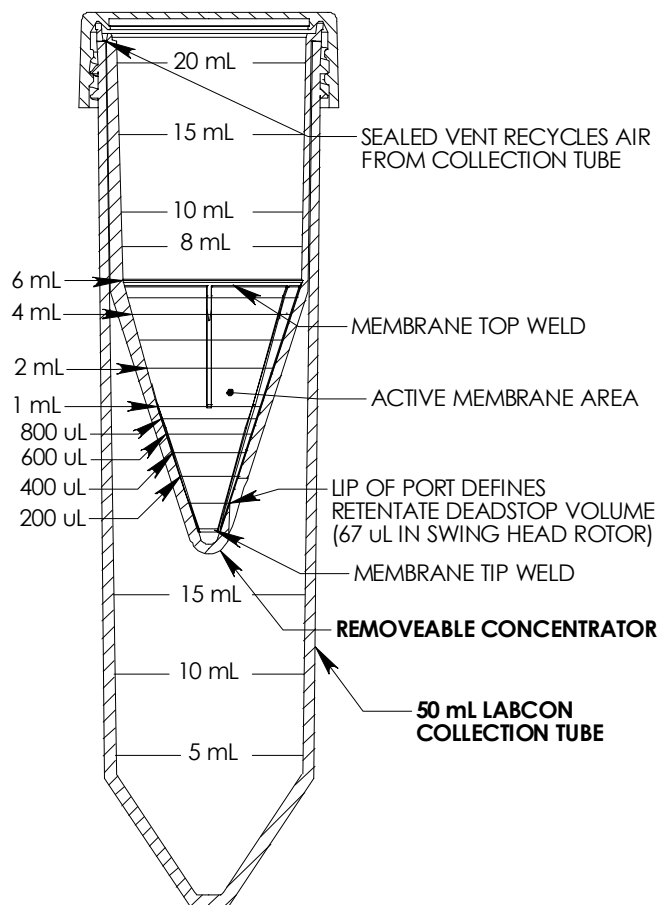
Active membrane area: 12 cm²
 Collection tube: Diameter, OD: 29.2 mm
 Length (incl. cap): 118.2 mm
 Filter: Length (filter tip to top flange): 68 mm
 Diameter (below top flange): 26.8 mm

Environmental Resistance

Temperature: 34.7 °C, 120 °F, max. Do not autoclave

Limit of pH: 1 to 14

AXIAL SECTION OF APOLLO 20 mL CONCENTRATOR ASSEMBLY



Chemical Compatibility

Common chemicals (√ = acceptable; **X** = *not recommended*)

Acids and Bases

Acetic acid (10%)	√	Hydrochloric acid (1.0N)	√	Sodium hydroxide (0.1N)	√
Ammonium hydroxide (10%)	√	Lactic acid (50%)	√	Sodium hydroxide (2.5N)	X
Formic acid (70%)	√	Perchloric acid (5%)	√	Trichloroacetic acid (10%)	√
		Phosphoric acid (30%)	√		

Organic Solvents, Miscellaneous Chemicals

Acetone	X	Dithiothreitol ((0.1 M)	√	Propanol (70%)	√
Acetonitrile (40% in 1% TFA)	√	Ethanol (70%)	√	Pyridine	√
Acetonitrile	√	Ethyl acetate	√	PyroCLEAN™	√
Alconox™ (1%)	√	Formaldehyde (5%)	√	Sodium carbonate (20%)	√
Ammonium sulfate (50%)	√	Formamide	√	Sodium chloride (2M)	√
Benzene	X	Glycerin	√	Sodium deoxycholate (5%)	√
n-Butanol	√	Guanidine HCl (6M)	√	Sodium dodecyl sulfate (0.1M)	√
CAPS (250 mM, pH 11.0)	√	Guanidine thiocyanate	√	Sodium thiocyanate (3M)	√
Carbon Tetrachloride	X	Imidazole (1M)	√	Terg-A-Zyme™ (1%)	√
CHAPS (100 mM)	√	Lubrol PX (0.1%)	√	Tetrahydrofuran	X
Chloroform	X	Mercaptoethanol (0.1M)	√	Toluene	X
Diethyl pyrocarbonate (0.2%)	√	Methanol	√	Tris buffer (1M, pH 8.2)	√
Dimethyl formamide	√	Nonidet P-40® (2%)	√	Triton X-100™ (0.002M)	√
Dimethyl sulfoxide	√	Phenol (1%)	√	Tween-20™	√
Dioxane	√	Phosphate buffer (1M, pH 8.2)	√	Urea (8M)	√
		Polyethylene glycol (PEG400, 10%)	√		

Some of the recommended chemicals listed above may affect membrane performance, thereby altering the recoveries, passage, and /or spin times. Alconox is a registered trademark of Fabric Chemicals, Co. Nonidet P-40 is a registered trademark of Shell Oil Co. Terg-A-Zyme is a registered trademark of Rohm and Haas Co. Tween is a registered trademark of Atlas Powder Co.

HOW TO USE THIS PRODUCT

Preparations

Make sure it will fit in your centrifuge

Prepare a 50 mL carrier accepting a 118 mm length tube in centrifuge. Either fixed angle or swing head rotors can be used, although performance is better in a swing head. Check clearance of tube to both swing mechanism and rotor cover or centrifuge lid.

Make sure you have chosen the right device for your application

Select a device with a retention rating equal to or smaller than the MW of the macromolecule to be concentrated (see Table I). The membrane Quantitative Molecular Weight Limit (QMWL) rating is engraved near the top lip. Insert the concentrator into the filtrate collection tube.

If glycerin removal is required

Add 15 mL clean water or buffer. Place device assembly into the rotor and counterbalance with a similar device or tube of the same weight. Spin at 8500 or maximal permitted rotor rcf to produce >5 mL filtrate. Shake water out of device and collection tube, and then replace the device in the tube.

Operation

1. Add sample and cap tube snugly.

An internal vent hole near the lip permits air from the collection tube to pass into the concentrator to maintain maximal flow without release of aerosols.

2. Place assembly into rotor.

Counterbalance with a similar device or tube of the same weight and spin. Note specified centrifugal force limits and observe max. relative centrifugal force rating for the rotor.

3. Spin for the required time (see Table II)

Spin at the suggested speed to achieve the desired concentration factor. To exchange microsolutes by diafiltration, decant filtrate, add 1 mL buffer to device, vortex mix, and then fill device to capacity. Concentrate and dilute until desired solute removal is achieved. If your application will allow a concentration factor of greater than 500x, 100% salt or solute removal is possible in a single spin.

4. Harvest retentate

Use a 200 μ L or smaller pipette tip to avoid damage to the membrane near the tip of the concentrator. Gently aspirate the retentate directly from the concentrator, holding the pipette at a slight angle to permit flow into the tip.

Precautions

- **Avoid scraping membrane skin** with pipette tip when adding or decanting. Exceeding the maximum centrifugal force limits specified above may cause retentate leakage.
- **Avoid excessive rcf with membranes of larger QMWL ratings.** With linear nucleic acids, or when partially separating smaller proteins from larger ones, maximal selectivity is obtained at filtration velocities <1 mm/min. In Apollo 20 mL, this corresponds to filtration rates <1.2 mL/min.
- **For best recovery, remove retentate in <10 min.** Upon standing, wicking by the spun, partly desiccated membrane can cause continued filtration, further reducing retentate volume. For retentate volumes <50 μ L, mass recovery is improved by adjusting volume with buffer to about 50 μ L before recovery, and/or by subsequently adding 50-200 μ L of buffer to the device, mixing by vortexing or aspirating into and out of the tip several times and recovering the wash as well.
- **To clean devices, vortex with 1.5 mL or sonicate with 5 mL of surfactant.** Discard. Vortex then rinse several times with water or buffer. Refrigerate, filled with several mL of buffer, water, or alcohol and tightly capped to avoid drying of the membrane skin and permanent loss in flow rate.

TYPICAL PERFORMANCE

Table I: Membrane Retention

		Retention of diafiltered solute				
QMWL (Quantitative Molecular Weight Limit) >95% retention of globular proteins, Daltons		5,000	10,000	30,000	70,000	150,000
Equivalent Nominal Molecular Weight Limit ("Cutoff") >90% Rating		5,000	8,000	10,000	30,000	100,000
Challenge Solute	Molecular Wt.					
1.0 A ₂₇₀ d(pC) ₂₁ oligonucleotide	10k Da, linear	n.a.	77%	15%	3%	2%
1.0 A ₂₇₀ d(pC) ₂₄₋₃₄ oligonucleotide	11-16k Da, linear	n.a.	93%	71%	50%	14%
200bp DNA	130k Da, linear	99%	99%	98%	n.a.	n.a.
0.25 mg/mL bovine cytochrome-c	12k Da, globular	99%	99%	10%	n.a.	n.a.
1 mg/mL alpha-chymotrypsinogen	25k Da, globular	99%	99%	94%	4%	n.a.
1 mg/mL bovine carbonic anhydrase	29k Da, globular	99%	99%	98%	n.a.	n.a.
1 mg/mL ovalbumin	46k Da, globular	99%	99%	98%	17%	n.a.
1 mg/mL bovine serum albumin	67k Da, globular	99%	99%	99%	98%	29%
1 mg/mL alcohol dehydrogenase, yeast	150k Da, globular	n.a.	n.a.	n.a.	97%	n.a.
1 mg/mL bovine IgG	150k Da, globular	n.a.	n.a.	n.a.	91%	n.a.
1 mg/mL bovine γ globulin	175-900k Da, globular	n.a.	n.a.	n.a.	96-99%	n.a.
1 mg/mL apoferritin, horse heart	443k Da, globular	n.a.	n.a.	n.a.	>99%	97-99%
0.5 mg/mL bovine thyroglobulin	669k Da, globular	n.a.	n.a.	n.a.	n.a.	97-99%

All protein dissolved in pH 7.4, 0.01M Phosphate buffered saline solution (PBS).

Table II: Time to Concentrate

Actual conditions will vary with details of initial solution temperature, concentration, and protein characteristics, but the table below can be used to provide an estimate of spin time (more detailed data is available at www.orbio.com).

Device	Solution	Vol.	Rotor	RCF	Time (min)	Conc. factor
10k Da	BioCel Water	20 mL	Swing head	4,500	30	250X
10k Da	25 ug/mL equine cytochrome-c, 0.01M PBS	20 mL	Swing head	4,500	20	67X
10k Da	250 ug/mL equine cytochrome-c, 0.01M PBS	20 mL	Swing head	4,500	20	64X
10k Da	250 ug/mL equine cytochrome-c, 0.01M PBS	13.5 mL	34° angle	6,000	30	39X
10k Da	250 ug/mL equine cytochrome-c, 0.01M PBS	13.5 mL	34° angle	12,000	20	111X
10k Da	250 ug/mL equine cytochrome-c, 0.01M PBS	13.5 mL	34° angle	12,000	30	232X
30k Da	BioCel Water	20 mL	Swing head	4,500	10	100X
30k Da	0.5 mg/mL bovine serum albumin	20 mL	Swing head	4,500	20	150x

Ordering Information

Product Name	QMWL*	Identification	Qty/Pk	Order No.
5k Apollo 20 mL	5k Da	Sample pack	2 ea.	AP2000500
5k Apollo 20 mL	5k Da	Rack of filters in capped tubes	25 ea.	AP2000510
5k Apollo 20 mL	5k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP2000520
5k Apollo 20 mL	5k Da	Bulk bags of filters only	1000 ea.	AP2000531
10k Apollo 20 mL	10k Da	Sample pack	2 ea.	AP2001000
10k Apollo 20 mL	10k Da	Rack of filters in capped tubes	25 ea.	AP2001010
10k Apollo 20 mL	10k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP2001020
10k Apollo 20 mL	10k Da	Bulk bags of filters only	1000 ea.	AP2001031
30k Apollo 20 mL	30k Da	Sample pack	2 ea.	AP2003000
30k Apollo 20 mL	30k Da	Rack of filters in capped tubes	25 ea.	AP2003010
30k Apollo 20 mL	30k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP2003020
30k Apollo 20 mL	30k Da	Bulk bags of filters only	1000 ea.	AP2003031
70k Apollo 20 mL	70k Da	Sample pack	2 ea.	AP2007000
70k Apollo 20 mL	70k Da	Rack of filters in capped tubes	25 ea.	AP2007010
70k Apollo 20 mL	70k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP2007020
70k Apollo 20 mL	70k Da	Bulk bags of filters only	1000 ea.	AP2007031
150k Apollo 20 mL	150k Da	Sample pack	2 ea.	AP2015000
150k Apollo 20 mL	150k Da	Rack of filters in capped tubes	25 ea.	AP2015010
150k Apollo 20 mL	150k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP2015020
150k Apollo 20 mL	150k Da	Bulk bags of filters only	1000 ea.	AP2015031
		Rack of 25 ea tubes and caps for Apollo 20 mL	25 ea.	AP2000000
		Case of tubes & caps for Apollo 20 mL	500 ea.	AP20000

*Minimum protein molecular weight that has been found to be quantitatively (>95%) retained by the membrane when tested in an Apollo device, as determined by filtrate optical density.

To reorder, please refer to Product Numbers above.

Technical Assistance

Either call, fax, or e-mail us at the numbers below for help. Or visit us on the Internet at our World Wide Web site (www.orbio.com) for the most up-to-date technical information on the Apollo family of products.

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