Orbital Biosciences®

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.

Apollo 20 mL concentrators are disposable ultrafiltration devices for the concentration or purification of protein solutions. They are far superior to alternatives in simplicity, speed, and recovery. This is due to their unique conical design (US Patent 6,269,957; 6,357,601), increasing both area of hydrophilic membrane and sample size that, in turn, provide higher degrees of concentration possible in a single spin as well as better control of protein polarization and fouling at the membrane surface.

SPECIFICATIONS

Volumes

	Maximum Sample
34º angle rotor:	13.5 mL
With swing-head rotor:	20 mL

Total Volume In Concentrator & Filtrate Tube

Swing Head	34º Angle	Resulting Deadstop
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

35º angle rotor: 13,000 rcf , 3,500 rcf 150k
Swing rotor: 4,500 rcf rotor maximum, 3,500 rcf 150k

Materials

Membrane: Regenerated cellulose on polyester nonwoven support. Contains glycerol.
Concentrator, collection tube and cap: Polypropylene

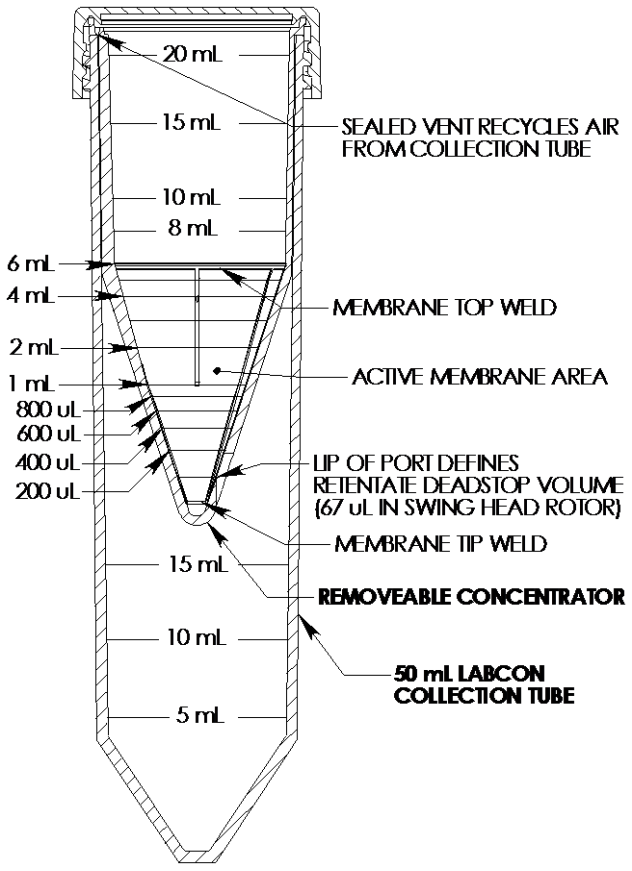
Dimensions

Active membrane area: 12 cm^2
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 ºC, 120 ºF, max. Do not autoclave
Limit of pH: 1 to 14

AXIAL SECTION OF APOLLO 20 mL CONCENTRATOR ASSEMBLY



Membrane Seal Integrity

Apollo concentrators are 100% air tested to exclude devices with gross defects in the membrane thermal weld. Each lot is sampled and challenged with a retained protein to ensure average retentate recovery >90%. Occasional wrinkles or creases in the membrane do not cause devices to fail to meet specified flow or retention.

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-Zymne™ (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 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 recommended 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 maximum 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 microsolute by diafiltration, decant filtrate, add 1mL 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 µL or smaller pipette tip to avoid damage to the membrane near the tip of the concentrator. Viewing from above, slide tip down the groove formed by the vertical membrane seam. Gently aspirate the retentate directly from the concentrator, holding the pipette at a slight angle to permit flow into the tip. For added recovery, subsequently add an ~ equal volume (50-200 µL) of buffer as a wash to the device, optionally allowing to stand for up to15 minutes before aspirating the wash as well.

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 retention 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 µL, mass recovery is improved by adjusting volume with buffer to about 50 µL before recovery.

- ***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

Molecular Weight Cut Off MWCO, >90%:		**9k**	**20k**	**150k**
Challenge Solute	**MWt., Da**	**% Retention**		
1mg/mL ubiquitin	6.7k	80		
0.25 mg/mL bovine cytochrome-c	12k	**>94**	>80	
0.25 mg/mL equine myoglobin	17k	**>94**	>86	
1 mg/mL alpha-chymotrypsinogen	25k		**>90**	
1 mg/mL ovalbumin	46k	**>94**	**>94**	<35
1 mg/mL bovine serum albumin	69k		**>94**	<75
1 mg/mL bovine IgG	150k			**>90**
1 mg/mL bovine γ globulin	175-900k			**>90**
1 mg/mL apoferritin, horse heart	443k			**>94**

All proteins 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.

Device	Solution	Vol.	Rotor	RCF	Time (min)	Conc. factor
9k Da	**250 ug/mL equine cytochrome c**	**20 mL**	**Swing head**	**4.5k**	**45**	**150X**
20k Da	**1 mg/mL ovalbumin**	**20 mL**	**Swing head**	**4.5k**	**30**	**75x**
150k Da	**1 mg/mL gamma globulins**	**20 mL**	**Swing head**	**2k**	**25**	**200x**

Ordering Information

Product Name	MWCO Da	Identification	Qty/Pk	Order No.
9k Apollo 20 mL	9k	Sample pack	2 ea.	AP2000900
9k Apollo 20 mL	9k	Bag of 8 filters in capped tubes	8 ea.	AP2000904
9k Apollo 20 mL	9k	Rack of filters in capped tubes	25 ea.	AP2000910
9k Apollo 20 mL	9k	Bag of 100 filters only	100 ea.	AP2000942
20k Apollo 20 mL	20k	Sample pack	2 ea.	AP2002000
20k Apollo 20 mL	20k	Bag of 8 filters in capped tubes	8 ea.	AP2002004
20k Apollo 20 mL	20k	Rack of filters in capped tubes	25 ea.	AP2002010
20k Apollo 20 mL	20k	Bag of 100 filters only	100 ea.	AP2002042
150k Apollo 20 mL	150k	Sample pack	2 ea.	AP2015000
150k Apollo 20 mL	150k	Bag of 8 filters in capped tubes	8 ea.	AP2015004
150k Apollo 20 mL	150k	Rack of filters in capped tubes	25 ea.	AP2015010
150k Apollo 20 mL	150k	Bag of 100 filters only	100 ea.	AP2015042
		Rack of 25 ea tubes and caps for Apollo 20 mL	25 ea.	AP2000000
		Case of tubes & caps for Apollo 20 mL	500 ea.	AP2000002

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.orbiitalbiosciences.com) for the most up-to-date technical information on the Apollo family of products.

Orbital Biosciences, LLC

4 Winsor Lane • P.O. Box 304 • Topsfield, MA 01983 • USA

Tel. 978-887-5077• FAX 978-887-2906 • e-mail: orbio@shore.net