Orbital Biosciences®

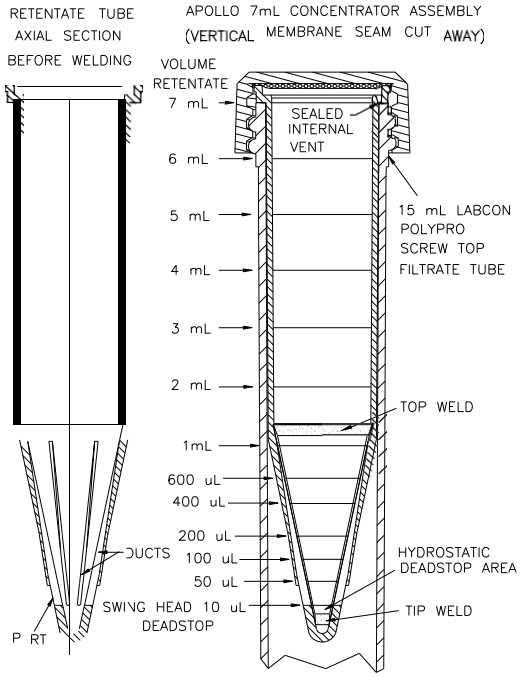
USER GUIDE

Apollo® 7mL

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 7 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), providing a high ratio of non- adsorptive 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 7 mL has the largest sample volume capacity for its centrifuge rotor size.



SPECIFICATIONS

Volumes

	Maximum Initial Sample	Deadstop
With 35º angle rotor:	5 mL	3 µL
With swing-head rotor:	7 mL by decanting filtrate	10 µL
	6 mL without decanting	10 µL

Total Volume Added to Sample + Filtrate Tubes

Swing Head	35º	Deadstop
NA	<= 5.7 mL	3 µL
<= 6.2 mL	6.1 mL	10 µL
6.45 mL	6.25 mL	20 µL
6.85 mL	6.6 mL	50 µL
7.2 mL	6.9 mL	100 µL
7.6 mL	7.35 mL	200 µL
8.25 mL	8.05 mL	500 µL
9.0 mL	8.75 mL	1000 µL

Maximum Centrifugal Force

35º angle rotor: 8,500 rcf (200 psi with 5 mL)

Swing-head rotor: 4,500 rcf typical rotor maximum

Materials

Membrane: Regenerated cellulose skin on polyester nonwoven support. Membranes contain glycerol.

Sample reservoir, vial and cap: Polypropylene

Dimensions

Active membrane area: 5.2 cm^2

Tube: Diameter, OD: 16.8 mm, 0.66 in (typical)

Length (incl. cap): 123.4 mm, 4.86 in (typical)

Filter: Diameter: 14.3 mm, 0.56 in (typical)

Length (filter tip to top): 73.4 mm, 2.89 in (typical)

Environmental Resistance

Temperature: 34.7 ºC, 120 ºF, max. Do not autoclave

Limit of pH: 1 to 14

Membrane Seal Integrity

Apollo concentrators are 100% air tested to exclude devices with gross defects in the membrane thermal weld. Each production lot is then sampled and challenged with a retained protein to confirm average retention of at least 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 s

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 15 to 17 mL carrier accepting a 124 mm length tube in centrifuge, either fixed angle or swing head rotors can be used. 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). Insert the device into the filtrate collection tube.

If glycerin removal is required

Add 5 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 >4 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 filter 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 microsolute by diafiltration, decant filtrate, and refill with buffer, mixing retentate by repeated aspiration with the pipette tip held near the top of the cone to avoid scraping the membrane. 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 pipette tip small enough to reach the bottom of the device. Remove Apollo from the collection tube and hold it up to a light. The meniscus may be seen in the conical section through the viewing port formed between the vertical membrane edges. The tip of the recovery pipette is easily seen when it touches the bottom of the device retentate chamber.

Precautions

- ***Avoid scraping membrane skin*** with pipette tip when adding or decanting. Exceeding the maximum centrifugal force limits specified above may cause retentate leakage. With linear nucleic acids, maximal selectivity is obtained at filtration velocities <1mm/min. In Apollo 7 mL, this corresponds to filtration rates <0.5 mL/min. For most selective retention of nucleic acids and removal of primers and oligonucleotide when concentrating and diafiltering DNA and RNA, reduced rcf should be used.

- For best recovery***, remove retentate in <10 min***. Upon standing, wicking can cause it to spread upward and continue to filter, further reducing retentate volume. For retentate volumes <10 µL, mass recovery is improved by adjusting volume with buffer to about 10 µL before recovery, and/or by subsequently adding 20-100 µL of buffer to the device, mixing into and out of the tip several times and recovering the wash as well.

- ***To clean devices, vortex or sonicate them*** with 2 – 3 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

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

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.

Device	Solution	Vol.	Rotor	RCF	Time (min)	Conc. Factor
9k Da	0.25 mg/mL bovine cytochrome c	5 mL	Swing head	4,500	35	175x
20k Da	1 mg/mL ovalbumin	5 mL	Swing head	4,500	25	175x
150k Da	0.5 mg/mL bovine thyroglobulin	5 mL	Swing head	2.000	20	500x

Ordering Information

Product Name	MWCO, Da	Identification	Qty/Pk	Order No.
9k Apollo 7 mL	9k	Sample pack	2 ea.	AP0700900
9k Apollo 7 mL	9k	Bag of 12 filters in capped tubes	12 ea.	AP0700906
9k Apollo 7 mL	9k	Rack of filters in capped tubes	25 ea.	AP0700910
9k Apollo 7 mL	9k	Bag of 100 filters only	100 ea.	AP0700942
20k Apollo 7 mL	20k	Sample pack	2 ea.	AP0702000*
20k Apollo 7 mL	20k	Bag of 12 filters in capped tubes	12 ea.	AP0702006*
20k Apollo 7 mL	20k	Rack of filters in capped tubes	25 ea.	AP0702010*
20k Apollo 7 mL	20k	Bag of 100 filters only	100 ea.	AP0702042*
150k Apollo 7 mL	150k	Sample pack	2 ea.	AP0715000
150k Apollo 7 mL	150k	Bag of 12 filters in capped tubes	12 ea.	AP0715006
150k Apollo 7 mL	150k	Rack of filters in capped tubes	25 ea.	AP0715010
150k Apollo 7 mL	150k	Bag of 100 filters only	100 ea.	AP0715042
		Rack of 25 tubes and caps for Apollo 20 mL	25 ea.	AP0700000
		Case of tubes & caps for Apollo 20 mL	500 ea.	AP0700002

- Limited availability

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.orbitalbiosciences.com) for the most up-to-date technical information on the Apollo 7 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