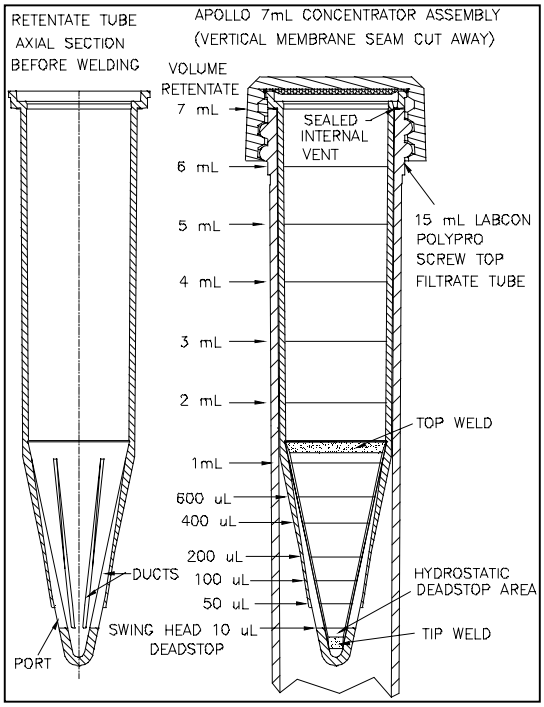
APOLLO® 7 mL Technical Data Bulletin

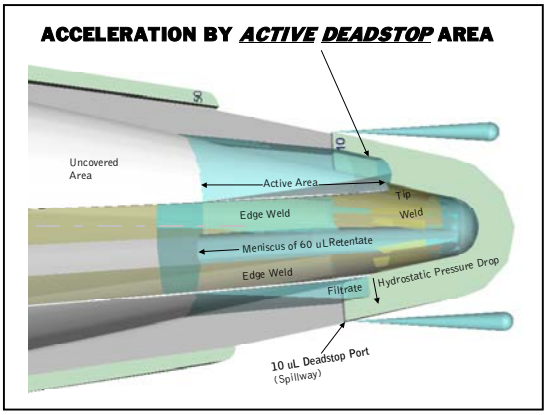
High-Performance Concentrators For Quantitative Protein Separations

This product is offered for research use only. Not for clinical use nor for preparation of fluids to be used for human injection.

Optimized for Performance

The high-performance ultrafiltration of Apollo 7 mL is the result of accelerated conical focusing of retained proteins combined with improved use of regenerated cellulose membrane (US Patents 6,269,957; 6,357,601; PCT pending). The larger active membrane area reduces protein exposure to sticky plastic surfaces. The conical membrane shape extends below the level of the permeate ports. The active filtration area in the region below the ports supports a high filtration rate as the deadstop volume is approached. When the sample meniscus reaches the level of the permeate ports, hydrostatic pressure equalizes across the active membrane below the ports and filtration stops.





The steep angle of the cone assures continuous removal of denser retained protein as it slides down into the recovery cup formed at the tip of the cone. This depolarization improves performance compared to designs with membrane placed at shallower angles to the centrifugal field. Apollo focuses all the retentate into a single drop with minimal bounding surfaces. This facilitates direct pipette recovery without the need for an inverted spin recovery step. The Apollo conical design also accepts a larger sample volume for a given tube size than other devices which constrict the membrane in a separate thin lower chamber.

Applications

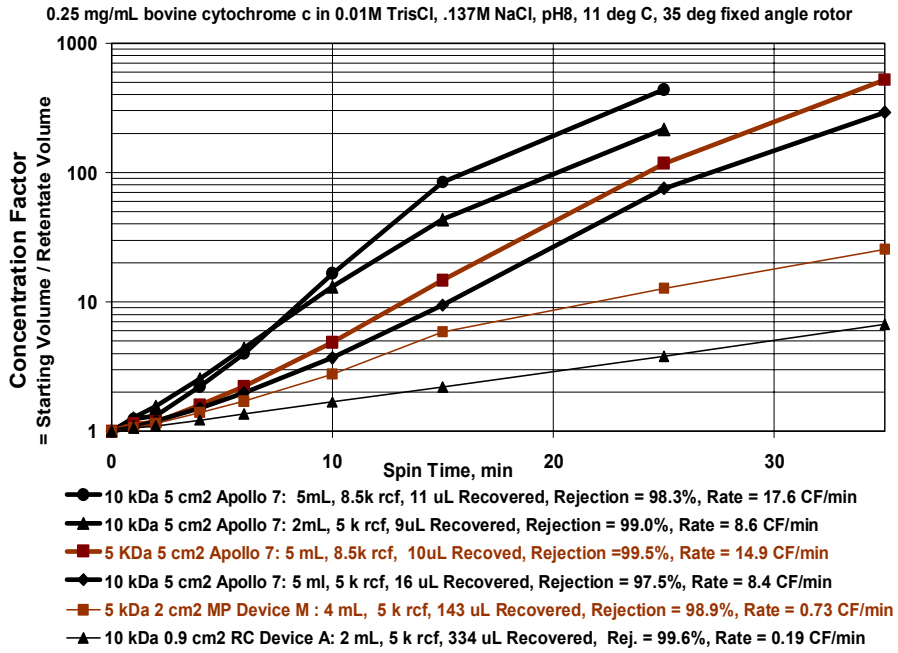
- Protein concentration and purification
- Desalting and buffer exchange
- Removal of unincorporated label
- Removal or exchange of cofactors or metals
- Buffer replacement following protein elution from ion-exchange or HIC columns
- Concentration of peaks following size exclusion chromatography
- Biosafe concentration of virus from tissue culture media, with passage of media protein

Performance Data

Protein Concentrating Rate

In **Figure 1**, concentration rates for Apollo 7 mL containing 5.2 cm^2 of a 10,000 Da-rated regenerated cellulose (RC) membrane are compared to that of a 4 mL capacity high-flux 2 cm^2 modified polysulfone (MP) commercial concentrator (Device M), and that of a 2 mL capacity high-recovery 0.9 cm^2 RC commercial concentrator (Device A). Both were selected with membranes of comparable quantitative rejection of cytochrome c. Device weights were followed with successive spins at 5,000 rcf, the maximum rating for Device A. The final recoverable retentate volume in each device was measured with an adjustable microvolume pipette as soon as possible after stopping the rotor and used to correct the weight estimates of volume in calculating the concentration factor curves. Apollo devices with 5 mL were also run at 8,500 rcf. By plotting log of concentration factor vs. time, a nearly linear measure of concentration rate is seen which is more revealing of performance than are asymptotic volume-vs-time profiles. The higher concentration rate of the Apollo design and its ability to provide up to 1000-fold concentration without filtering to dryness are evident. Apollo 7 mL demonstrates an overall rate of 8.4 CF/min in reducing 5 mL of 0.25 mg/mL cytochrome c to 16 μL in 35 min at 5,000 rcf, and 8.6 CF/min in concentrating 2 mL to 9 μL in 25 min. This rate is an order of magnitude greater than the 0.73 CF/min obtained with Device M. It is 45 times faster than Device A when Apollo is run at the maximum speed of Device A. Note the further performance improvement by operating Apollo at its maximum suggested speed of 8,500 rcf. The rate increases to 17.6 CF/min, concentrating 5 mL to 11 μL in only 25 min.

Figure 1: Fixed Angle Rotor Protein Concentration Rates



In **Figure 2**, initial buffer rates and protein concentration rates of Apollo in a swing head rotor are compared to a recently-introduced commercial polyethersulfone membrane device, designated PES 6, having similar retention rating and capacity. The bold 600x horizontal line and 200x dashed lines show the calculated maximal CF at deadstop of the respective devices with 6mL starting volumes. The 10k Da Apollo unit is seen to pass the competitor at 15 minutes as both devices reach 60 μL or 100x. From there, the hydrostatic deadstop brings Apollo to 92% of maximal concentration in another 14 minutes while the other device reaches only 58% of its maximal in the same time. The 10k Da Apollo 7 mL produces four times more CF in the same time than the 5k Da PES6 device of comparable cytochrome retention. Apollo's regenerated 5k Da and 10k Da cellulose membranes retain about 90% of their buffer flux up to 100x concentration (up to 25 mg/mL) of cytochrome, demonstrating that this sticky hydrophobic protein does not foul the cellulose skin. By contrast, the cytochrome c rate of the 5k Da PES6 device seen here is only half that the initial buffer rate of the same device.

Figure 2: 6mL Swing Head Concentration Rates of 5 and 10k Da Devices

4500 rcf, pH 7.4 phosphate buffered saline (PBS) +/- 0.25mg/mL beef heart cytochrome c

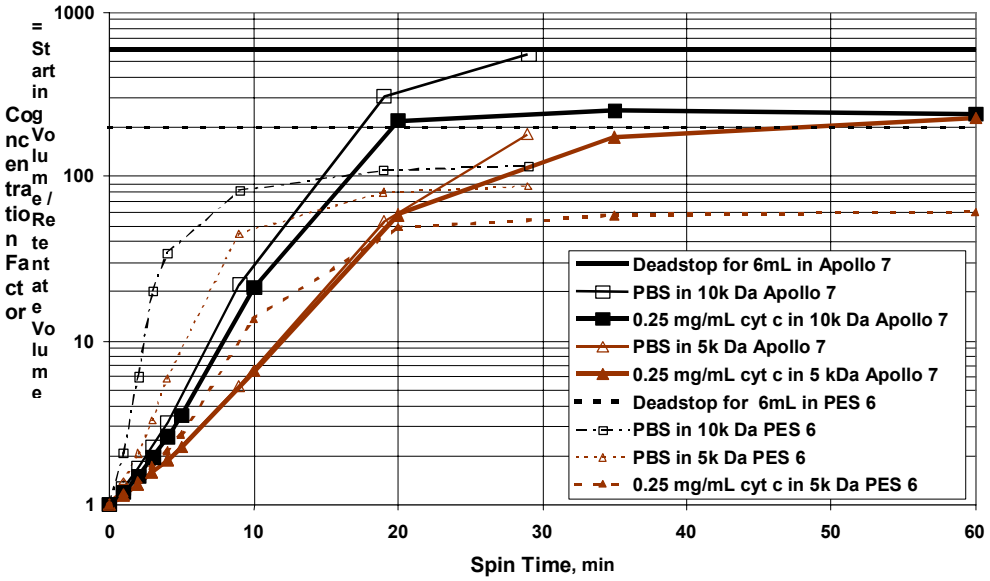


Figure 3 compares the performance of the 30k Da and 70k Da Apollo devices in concentrating 0.5 mg/mL bovine albumin to the initial buffer flux of the same devices. Both devices provide retentate concentrations above 150 mg/mL of albumin in 15 minutes.

Figure 3: 6mL Swing Head Concentration Rates of 30 & 70k Da Apollo 7

4500 rcf, pH 7.4 phosphate buffered saline (PBS) +/- 0.5mg/mL Fr V albumin (BSA)

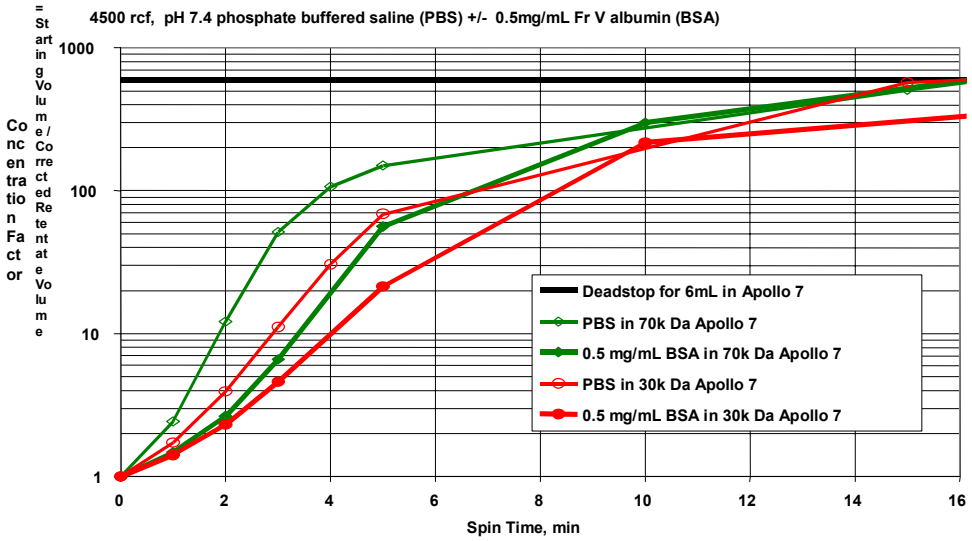
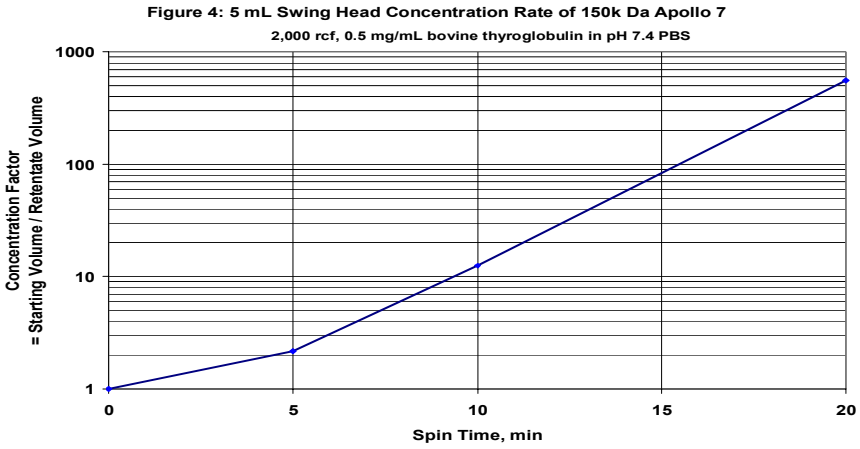


Figure 4 shows the rate of the 150k Da Apollo 7 mL in concentrating 0.5 mg/mL bovine thyroglobulin (669k Da) at reduced rcf (2,000), which might be used to improve selectivity of removal of media protein from cultured virus.



Recovery of Dilute Cytochrome c

To challenge the retentate recovery of dilute protein from Apollo 7 mL aggressively, a study was performed with the lowest possible concentration of bovine heart cytochrome c which can be directly measured spectrophotometrically by redilution to only 0.5 mL. A stock solution of 0.25 mg/mL was prepared in pH 7.4, 0.01M phosphate buffered normal saline (PBS). 12 μg were added to 5 mL of buffer (2.4 μg/mL) in each of six devices containing a 5,000 Da-rated membrane (to maximize retentivity). This is a significant recovery challenge, representing 2.2 μg of protein per cm^2 of active membrane in Apollo 7, roughly just one monolayer of protein compared to the active membrane area of the device.

Results are presented in **Table I**. The average concentration factor was 921 +/- 322. This represents a range from a high of 1351x for the device with 3.7 μL final volume to a low of 400x for the device with 12.5 μL final volume. As would be expected from film wetting, the 12.5 μL retentate had the highest retentate protein recovery of 96%, while the 3.7 μL volume contained only 58 % of the mass, with 28% more protein recovered in a 1 mL PBS rinse of the device.

Average retentate recovery was 79% +/-12%, with an average rinse recovery of 11% +/- 8%. **Combined retentate plus rinse recovery average was 90% +/- 6%. Overall mass balance was 99% +/- 11%. Device integrity was confirmed by very low filtrate recovery, the 17% maximum corresponding to just 2 mOD above the blank, equal to the noise level of the spectrophotometer. These results support superior recovery of highly dilute protein using Apollo 7 mL over that reported by the manufacturer of Device A.**

TABLE I

Recovery of 5 mL of 2.4 µg/mL Bovine Heart Cytochrome C

5k Da Apollo 7 mL, 8,000 rcf, 30 min, 15°C, pH 7.4 0. 01M PBS

Device	Pipet Recov. Vol. µL	Retentate Conc. Factor, -fold	Retentate Recov. %	1 mL Rinse Recov. %	Retentate + Rinse Recov. %	Filtrate Recov. %	Total Mass Recov. %
A020	4.5	1111	85	10	94	17	111
A014	6.55	763	76	11	87	17	104
A012	5.35	935	79	7	85	0	85
A008	5.18	965	80	7	87	0	87
A016	3.7	1351	58	28	86	13	99
A011	12.5	400	96	5	101	4	105
Avg.	**6.30**	**921**	**79**	**11**	**90**	**8**	**99**
Std. Dev.	**3.18**	**322**	**12**	**8**	**6**	**8**	**10**

Specifications

Volumes

	Max. initial sample vol.	Dead-stop volume
35° angle rotor:	5 mL	3 µL
Swing-head rotor:	7 mL decanting filtrate	10 µL
	6 mL without decanting	10 µL

Maximum centrifugal Force

35° angle rotor: 8,500 rcf (200 psi with 5 mL)
Swing rotor: 4,500 rcf rotor max.; < 8,500 rcf

Materials

Membrane: Regenerated cellulose
Sample reservoir, vial and cap: Polypropylene copolymer

Dimensions

Active membrane area: 5.2 cm^2
Collection tube:
Diameter, OD: 16.8 mm, 0.66 in
Length (incl. cap): 123.4 mm, 4.86 in.
Filter:
Length of Filter : 73.4 mm, 2.89 in.
Diameter: 14.3 mm, 0.56in

Environmental Resistance

Temperature: 34.7 °C, 120 °F, max. Do not autoclave. Limit of pH: 1 to 14 (See Chemical Compatibility)

Chemical Compatibility

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

Acids and Bases		Carbon Tetrachloride	***X***	Phosphate buffer (1M, pH 8.2)	√
Acetic acid (10%)	√	CHAPS (100 mM)	√	Polyethylene glycol	√
Ammonium hydroxide (10%)	√	Chloroform	***X***	(PEG400,10%)	
Formic acid (70%)	√	Diethyl pyrocarbonate (0.2%)	√	Propanol (70%)	√
Hydrochloric acid (1.0N)	√	Dimethyl formamide	√	Pyridine	√
Lactic acid (50%)	√	Dimethyl sulfoxide	√	PyroCLEAN™	√
Perchloric acid (5%)	√	Dioxane	√	Sodium carbonate (20%)	√
Phosphoric acid (30%)	√	Dithiothreitol ((0.1 M)	√	Sodium chloride (2M)	√
Sodium hydroxide (0.1N)	√	Ethanol (70%)	√	Sodium deoxycholate (5%)	√
Sodium hydroxide (2.5N)	***X***	Ethyl acetate	√	Sodium dodecyl sulfate	√
Trichloroacetic acid (10%)	√	Formaldehyde (5%)	√	(0.1M)	
Organic Solvents, Miscellaneous Chemicals		Formamide	√	Sodium thiocyanate (3M)	√
		Glycerin	√	Terg-A-Zyme™ (1%)	√
Acetone	***X***	Guanidine HCl (6M)	√	Tetrahydrofuran	***X***
Acetonitrile (40% in 1% TFA)	√	Guanidine thiocyanate	√	Toluene	***X***
Acetonitrile		Imidazole (1M)	√	Tris buffer (1M, pH 8.2)	√
Alconox™ (1%)	√	Lubrol PX (0.1%)	√	Triton X-100™ (0.002M)	√
Ammonium sulfate (50%)	√	Mercaptoethanol (0.1M)	√	Tween-20™	√
Benzene	***X***	Methanol	√	Urea (8M)	√
n-Butanol	√	Nonidet P-40® (2%)	√		
CAPS (250 mM, pH 11.0)	√	Phenol (1%)	√		

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.

Selection and Ordering of Devices

Protein Retention Rating

In contrast to conventional cutoff or Nominal Molecular Weight Limit >90% retentivity ratings used by other manufacturers, Apollo devices are more conservatively specified to retain >95% of proteins of the Quantitative Molecular Weight Limit (QMWL) rating, as shown in **Table II**.

Table II: Membrane Retention Data		**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		*4,000*	*8,000*	*10,000*	*30,000*	*100,000*
Challenge Solute	**Molecular Weight**					
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 yeast alcohol dehydrogenase	**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 spleen	**443k Da, globular**	**n.a.**	**n.a.**	**n.a.**	**>99%**	**98%**
0.5 mg/mL bovine thyroglobulin	**660k Da, globular**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**97- 99%**

*Minimum protein molecular weight quantitatively (>95%) retained by the membrane, as determined by filtrate optical density. All proteins were dissolved in pH 7.4, 0.01M Phosphate-buffered saline solution (PBS). Retention is expressed as the ratio of filtrate OD at deadstop to that of the starting sample.

Product Name	QMWL*	Identification	Qty/Pk	Order No.
5k Apollo 7 mL	5k Da	Sample pack	2ea.	AP0700500
5k Apollo 7 mL	5k Da	Rack of filters in capped tubes	25 ea.	AP0700510
5k Apollo 7 mL	5k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP0700520
5k Apollo 7 mL	5k Da	Bulk bags of filters only	1000 ea.	AP0715031
10k Apollo 7 mL	10k Da	Sample pack	2ea.	AP0701000
10k Apollo 7 mL	10k Da	Rack of filters in capped tubes	25 ea.	AP0701010
10k Apollo 7 mL	10k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP0701020
10k Apollo 7 mL	10k Da	Bulk bags of filters only	1000 ea.	AP0715031
30k Apollo 7 mL	30k Da	Sample pack	2ea	AP0703000
30k Apollo 7 mL	30k Da	Rack of filters in capped tubes	25 ea.	AP0703010
30k Apollo 7 mL	30k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP0703020
30k Apollo 7 mL	30k Da	Bulk bags of filters only	1000 ea.	AP0715031
70k Apollo 7 mL	70k Da	Sample pack	2ea.	AP0707000
70k Apollo 7 mL	70k Da	Rack of filters in capped tubes	25 ea.	AP0707010
70k Apollo 7 mL	70k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP0707020
70k Apollo 7 mL	70k Da	Bulk bags of filters only	1000 ea.	AP0715031
150k Apollo 7 mL	150k Da	Sample pack	2ea.	AP0715000
150k Apollo 7 mL	150k Da	Rack of filters in capped tubes	25 ea.	AP0715010
150k Apollo 7 mL	150k Da	10 racks, bulk bags of filters, tubes, caps	250 ea.	AP0715020
150k Apollo 7 mL	150k Da	Bulk bags of filters only	1000 ea.	AP0715031
		Rack of 25 ea tubes and caps for Apollo 20 mL	25 ea.	AP0700000
		Case of tubes & caps for Apollo 20 mL	500 ea.	AP07TC

Terms and Conditions

Call, Fax or Email to obtain pricing. Otherwise, payment terms are net 30 days. F.O.B shipping point is Topsfield, MA. Shipping, handling and taxes due will be added to the invoice. Where partial deliveries are made, payment will be due on the portion delivered. Orders are subject to acceptance by Orbital Biosciences at current prices.

Claims

Claims for shipping damage are to be made to the transportation company. Obvious damage apparent on delivery should be noted on the delivery receipt. If damage is found after unpacking, the shipping company should be notified within 48 hours of delivery, requesting the preparation of a "Bad Order" report, to ensure damage recovery from the carrier. New England BioGroup or Orbital Biosciences should also be informed within 10 days of receipt of damaged goods or shortages. Lost shipment claims should be made within 30 days of the original order.

Returns

All returns must be made bearing a returned goods authorization number obtained from Orbital Biosciences. To obtain credit or exchange, requests must be made within 10 days of receipt of order.

Warranty

Orbital Biosciences warrants the quality of its products to be free of defects when used under the labeled intended conditions, and will replace any of its products that are defective. Since Orbital Biosciences cannot control the conditions of use of its products, we do not warrant suitability or optimal results in any specific application, nor that use of our products will not infringe on any patents. Orbital Biosciences furnishes its products For Research Use Only, not for use on humans, upon the condition that the purchaser assumes all risks and liabilities and that neither the manufacturer nor the seller shall be liable for any loss or damage, direct or consequential, arising from the use or inability to use our products.

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