

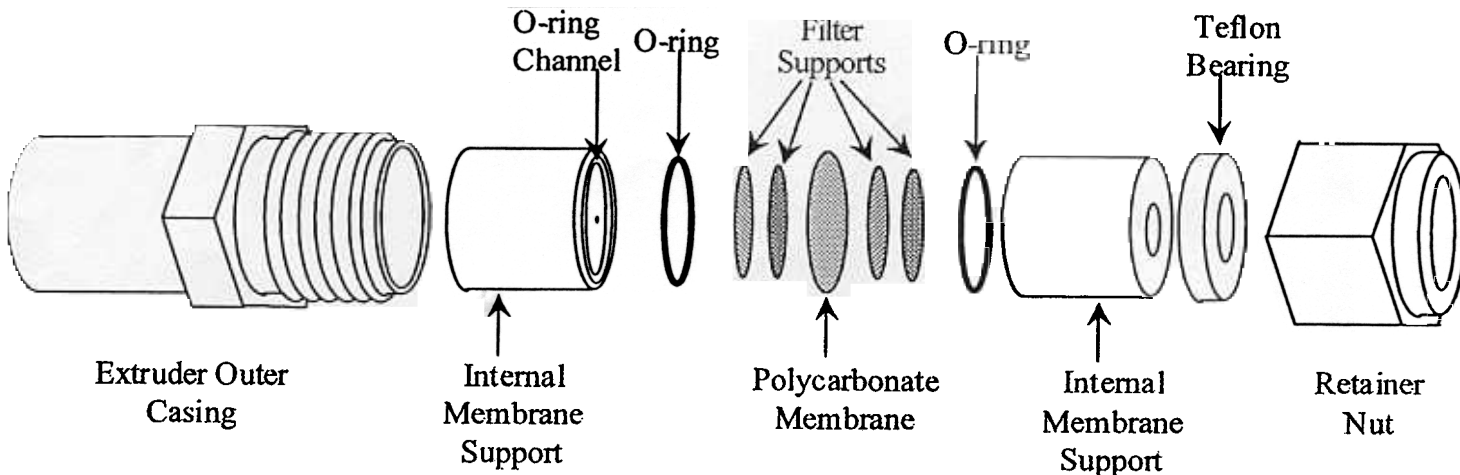
The Mini-Extruder

The *Avanti* Mini-Extruder allows researchers and scientists to prepare large, unilamellar vesicles by extrusion in an efficient, rapid manner. The optional heating block allows the extrusion of unilamellar vesicles at elevated temperatures, which is critical for the successful production of vesicles from phospholipids with a phase transition temperature (T_m) above room temperature. Constructed of stainless steel and Teflon, this new design allows rapid cleaning of all wetted parts, which reduces the “down-time” between production of vesicles from different lipid species. The Mini-Extruder is available for a fraction of the cost of a larger extruder. All parts for the Mini-Extruder are available from *Avanti*, and the Mini-Extruder comes with a 30 day workmanship guarantee.

The *Avanti* Mini-Extruder, Catalog No. 610000, set includes:

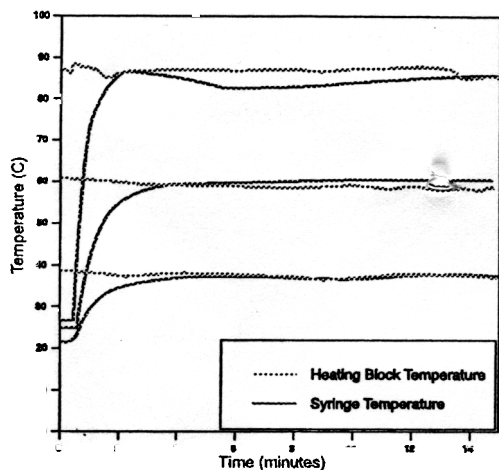
- (1) Mini-Extruder
- (1) Extruder Stand/Stabilizer Block
- (2) O-Rings
- (2) Gas Tight Syringes
- (100) Polycarbonate Membranes
- (100) Filter Supports

Sensitive to the divergent nature of each of our customers, and their unique applications for our products, *Avanti* stocks a variety of different Polycarbonate Membranes (from 0.03 μ m to 5 μ m) and two syringe sizes, 250 μ L and 1.0mL.



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Placing the Stabilizer block-extruder assembly on a hot plate achieves rapid temperature control for lipids that have transition temperatures above room temperature.

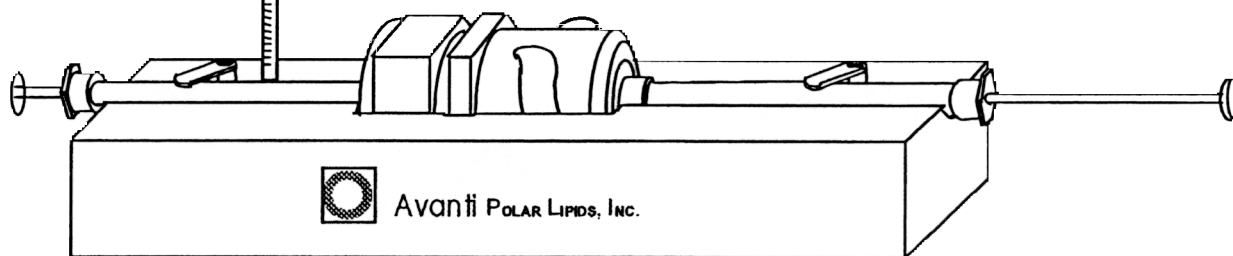
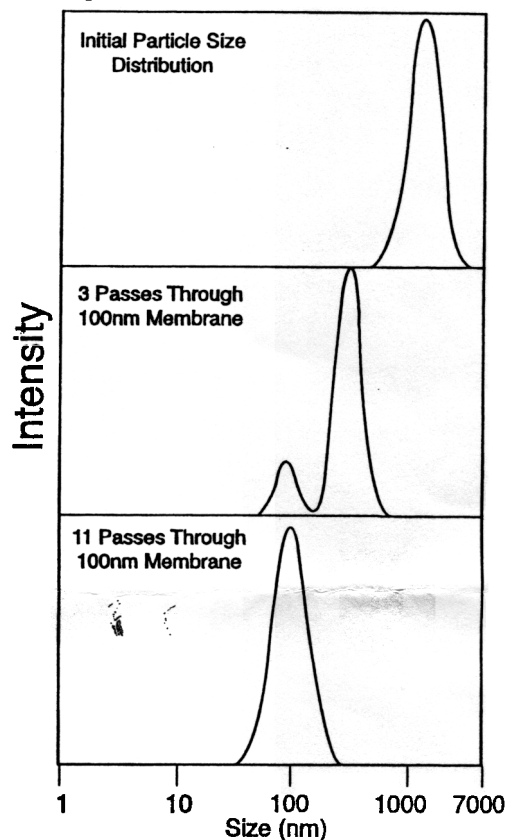


The particle size distribution of unilamellar vesicles prepared by extrusion is a function of the number of passes through the Extruder Membrane. Hydrated lipid solutions will initially form large, multi-lamellar vesicles. After the initial pass through a membrane, the particle size distribution will tend towards a bimodal distribution. After sufficient passes through the membrane, a unimodal, normal distribution is obtained. A minimum of eleven passes through the Extruder membrane is recommended for most lipids.

The particle size information in Figure 2 was obtained with a Coulter analyzer. The particle size analyzer was N4 Plus software package. The Coulter may be contacted at (800) 338-8830. A solution of 1,2-Dioleoyl-sn-glycero-3-Phosphocholine was made up to 25 mg/ml in DI water. The particle size distribution was measured for the material before extrusion and for 3 and 11 passes through a 100nm (0.1micron) membrane.

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Figure 2



References.

- Subbarao, N.K., R.I. MacDonald, K. Takeshita, and R.C. MacDonald. (1991). Characteristics of spectrin-induced leakage of extruded, phosphatidylserine vesicles. *Biochim Biophys Acta* 1063:147-54.
- MacDonald, R.C., R.I. MacDonald, B.P. Menco, K. Takeshita, N.K. Subbarao, and L.R. Hu. (1991). Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. *Biochim Biophys Acta* 1061:297-303.

The Mini-Extruder

Assembly Instructions

Before assembling the extruder for the first time, thoroughly clean all parts (except for the Polycarbonate Membranes and the Filter Supports) with a mild detergent solution, followed by rinsing with hot tap water and deionized or distilled water. Allow all parts to dry before assembling apparatus.

Important Note. The original Mini-Extruder design contains flat washers, and a bearing washer, that are not stainless steel and will rust if not thoroughly dried. Rinse with methanol or acetone and dry immediately. The all-teflon Mini-Extruder design replaces these components with a teflon bearing.

Assembly - refer to the diagram on page 1 to identify the parts in these instructions.

- 1) Place the 2 Internal Membrane Supports on a flat surface with the O-rings facing up.
- 2) Pre-wet 2 Filter Supports with DI water, or buffer, and place over orifice. The Filter Supports should adhere to the teflon orifice inside the O-ring inner diameter.
- 3) Insert the Internal Membrane Support, with the Filter Support, into the Extruder Outer Casing with the O-ring facing up.
- 4) Place 1 Polycarbonate Membrane in the Extruder Outer Casing over the Filter Support and O-ring. **Note.** The Polycarbonate Membrane is the thin, shiny disk - do not install one of the blue paper disks which separate the Polycarbonate Membranes.
- 5) Pre-wet a second pair of Filter Supports with DI water, or buffer, and place over orifice of remaining Internal Membrane Support.
- 6) Carefully place the second Internal Membrane Support into the casing (O-ring facing down) being careful not to twist the Membrane Support when it comes in contact with the Membrane.
- 7) If you have the original Mini-Extruder design, place the flat washers on either side of the bearing washer and insert into the Retainer Nut. For the all-teflon Mini-Extruder, place the teflon bearing into the Retainer Nut.
- 8) Place the Retainer Nut on the threaded end of the Extruder Outer Casing and tighten. **Tighten the Retainer Nut by hand just until it is finger tight; do not use a wrench.**

Important Note. Autoclaving the Teflon inserts is not recommended as slight distortion may occur ($\approx 0.002\text{mm}$). The use of ethylene oxide or gamma irradiation is suggested as an alternative. The syringes should be sterilized with ethanol.

Warranty

Avanti guarantees the apparatus free of defects of workmanship for a period of thirty (30) days. If you experience any problems due to workmanship during this time, please contact us to arrange an immediate replacement or exchange of the defective component.

The Mini-Extruder

Care and Cleaning Instructions for Unimetric Syringes

- 1) Immediately after use, carefully disassemble both Unimetric syringes.
- 2) Depending upon your application, rinse the syringes with either isopropyl alcohol, DI water, or a weak detergent solution. If a detergent solution is used, rinse the syringe immediately with copious quantities of DI water.
- 3) Rinse the syringes with DI water after cleaning.
- 4) Dry the syringes.
- 5) If it is necessary to use a sonic cleaner, only clean the needle with the sonic cleaner. Immediately after cleaning in the sonic cleaner, rinse with DI water and dry.
- 6) The teflon tip on the plunger may need to be replaced if the syringes are used at elevated temperatures for extended periods of time. Replacement teflon tips are available.

Important Note: Do not allow the syringes to come into contact with any solvents other than DI water or alcohol. Some organic solvents will interact with the glue holding the threaded insert onto the tip of the syringe possibly weakening the bond between the barrel and the insert. The syringes must not be soaked in any solvent for the same reason.

PARTS LIST							
Qty	Cat. No.	Item	Price	Qty	Cat. No.	Item	Price
	610000	Extruder, 2 syringes, PC membranes, 100 filter supports, holder/heating block	\$475.00		610023	Extruder, 2 syringes, 100 PC membranes, 100 filter supports	\$350.00
	610020	Extruder only	\$200.00		610024	Extruder holder/heating block	\$150.00
	610001	Membrane forceps 316SS	\$20.00		610014	X2 10mm Filter supports (100/pk)	\$44.85
	610002	0.03µm Polycarbonate membranes* 19mm	\$125.00		610015	X2 Gas-tight syringe 250µL	\$60.00
	610003	0.05µm Polycarbonate membranes* 19mm	\$110.00		610017	Gas-tight syringe 1.0mL	\$60.00
	610004	0.08µm Polycarbonate membranes* 19mm	\$105.00		610018	O-rings for extruder (2)	\$5.00
	610005	0.1µm Polycarbonate membranes* 19mm	\$120.00		610019	Teflon washer for extruder (1)	\$10.00
	610006	0.2µm Polycarbonate membranes* 19mm	\$77.75		610021	Replacement needle for 1.0mL syringe	\$10.00
	610007	0.4µm Polycarbonate membranes* 19mm	\$77.75		610022	Replacement needle for 250µL syringe	\$10.00
	610008	0.6µm Polycarbonate membranes* 19mm	\$77.75		610025	Teflon internal membrane support with O-rings (2)	\$70.00
	610009	0.8µm Polycarbonate membranes* 19mm	\$77.75		610026	Stainless steel extruder casing (1)	\$120.00
	610010	1.0µm Polycarbonate membranes* 19mm	\$77.75		610027	Replacement teflon plunger tip for 250µL syringe (3)	\$15.00
	610011	2.0µm Polycarbonate membranes* 19mm	\$77.75		610028	Replacement teflon plunger tip for 1.0mL syringe (3)	\$15.00
	610012	3.0µm Polycarbonate membranes** 19mm	\$77.75			Subtotal	
	610013	5.0µm Polycarbonate membranes* 19mm	\$77.75			Shipping	
* supplied in packs of 100 - **supplied in packs of 50							Total

Preparing Large, Unilamellar Vesicles by Extrusion (LUVET)

- 1) Prepare dry lipid mixture by lyophilization or evaporation.
- 2) Place the extruder stand/heating block onto a hot plate. Insert a thermometer into the well provided in the heating block. Switch the hot plate on, and allow to reach the desired temperature. Allow the temperature of the heating block to reach the desired value (approximately 15 minutes).
- 3) Hydrate lipid mixture using a suitable buffer for ≥ 30 min. The lipid suspension should be kept above the phase transition temperature of the lipid during hydration and extrusion. To increase the efficiency of entrapment of water-soluble compounds, one may subject the hydrated lipid suspension to 3-5 freeze/thaw cycles by alternately placing the sample vial in a dry ice bath and warm water bath.
- 4) Once the sample is fully hydrated, load the sample into one of the gas-tight syringes and carefully place into one end of the Mini-Extruder. Note: to reduce the dead volume, pre-wet the extruder parts by passing a syringe full of buffer through the extruder; discard the buffer. New syringes may have tight fitting parts; to facilitate extrusion, pre-wet syringe barrel and plunger with buffer prior to inserting plunger into barrel.
- 5) Place the empty gas-tight syringe into the other end of the Mini-Extruder. Make sure the empty syringe plunger is set to zero; the syringe will fill automatically as the lipid is extruded through the membrane.
- 6) Check the temperature of the heating block BEFORE placing the assembled extruder apparatus into the heating block. The temperature must be below 80°C , or the syringes will be damaged.
- 7) Insert the fully assembled extruder apparatus into the extruder stand. Insert the hex nut so that any two opposing apexes fall in the vertical plane. Use the swing-arm clips to hold the syringes in good thermal contact with the heating block.
NOTE: The extruder apparatus must be fully assembled before inserting in the heating block, otherwise it will be damaged.
- 8) Allow the temperature of the lipid suspension to equilibrate with the temperature of the heating block (approximately 5-10 minutes).
- 9) Gently push the plunger of the filled syringe until the lipid solution is completely transferred to the alternate syringe.
- 10) Gently push the plunger of the alternate syringe to transfer the solution back to the original syringe.
- 11) Repeat steps 9 & 10 a minimum of 4 times (total of 10 passes through membrane). In general, the more passes through the membrane, the more homogenous the lipid solution becomes.
- 12) The final extrusion should fill the alternate syringe. This is to reduce the chances of contamination with larger particles or foreign material.
- 13) After the final extrusion, remove the Mini-Extruder from the heating block.
- 14) Remove the filled syringe from the extruder and inject the lipid solution into a clean sample vial.
IMPORTANT: When removing syringes, pull the syringe straight out of the extruder; removing at an angle could result in cracking the syringe.
- 15) Store the vesicle preparation above the transition temperature of the lipid during the experiment. When not in use, store the vesicle solution at 4°C . Do not freeze. Vesicle solutions are not stable in aqueous media for more than 3-4 days when stored at 4°C . Storage of vesicle solutions at higher temperatures and $\text{pH} < 5$ or > 8 may reduce the lifetime of the vesicle suspension.
- 16) Clean apparatus thoroughly before using with a new lipid preparation.

Note on New Hamilton Syringes

In our continuing effort to resolve workmanship quality issues with the syringe for the Avanti Mini-Extruder, Avanti has contracted The Hamilton Company to provide syringes for the extruder. The syringes provided by The Hamilton Company have a larger diameter glass barrel than previous syringes. Therefore it is necessary to make minor adjustments to the syringe holding clips on the extruder heating block/stand to accommodate the larger syringes. Using a hex wrench, slightly loosen the hex screws which secure the syringe holding clips until they will easily pass over the syringe barrel, making sure that sufficient pressure is applied to the barrel to provide complete contact with the heating block/stand for heat transfer. Also, if the syringes do not lie flat in the heating block/stand syringe channel, rotate the syringe in the extruder one-half turn clockwise or until the syringe lies flat in the syringe channel (see photos).

Important: never rotate the syringe in the extruder counterclockwise as this will loosen the needle assembly from the glass barrel causing sample leakage.

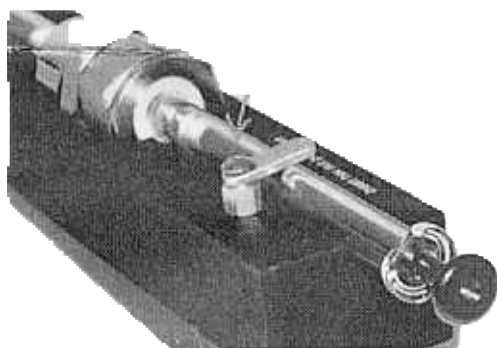


If you have any questions or need additional assistance, please do not hesitate to contact our Technical Support group at (205) 663-2494 or technical@avantilipids.com.

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