Dear Scientist,

Congratulations on your new purchase of a LIPEX™ Extruder. Each unit sold by Northern Lipids is made using high quality stainless steel components and is individually inspected and pressure-tested to ensure optimal performance and safety during operation.

As part of the testing procedure, vesicles of egg phosphatidylcholine (EPC) are made according to standard procedures known in the field. These multilamellar vesicles (MLVs) are passed through the Extruder according to the procedures described in the operating manual using two polycarbonate filters with a pore size of 100nm. The resulting unilamellar vesicles (LUVs) are analyzed by employing laser-based quasi-elastic light scattering techniques and a printout of the size distribution analysis is included in a test certificate supplied with each unit. The document shows that the Extruder supplied can produce vesicles of a size within our standard acceptance criteria (± 20% of the filter pore size).

Each unit is pressure tested through vessel pressurization, and calibration of the safety relief valve. The vessel is tested for leaks by assembling the Extruder with a pressure resistant sealing disc in place of the extrusion filter and pressurizing the Extruder to 1000 psigg (6850 kPa) while the unit is immersed in water. The Extruder is closely monitored during pressurization and passes the test only if gas leaks are absent over the full pressurization range (0 – 1000 psig / 0 – 68950 kPa). The safety relief valve on the associated pressure line is adjusted to vent nitrogen gas at pressure in excess of 800 psig (5440 kPa). As a final check to ensure that all parts are operational the Extruder is reassembled with all necessary components and the unit is pressurized until nitrogen gas vents from the relief valve. Upon passing inspection, the relief valve is locked into position and the Extruder is approved for sale.

We sincerely hope that you enjoy using your new equipment and that you have years of success in liposome production.

Sincerely,

Your NLI Extruder Team

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1 Introduction

Congratulations on your purchase of a LIPEX™ Extruder. The LIPEX™ Extruder is capable of generating a homogeneous population of unilamellar liposomes ranging from ~ 50 – 400 nm when assembled with the appropriate filters. It has been designed so that it is both easy to operate and to clean. All LIPEX™ Extruders sold are assembled and tested under standard operating procedures, quality assured and are ready to use upon receipt. It need only be attached to a regulator or a standard nitrogen gas cylinder. A Matheson Model 3 high-pressure regulator (in Canada, Model 3040-580) with a ¼ inch NPT male outlet is recommended. However, any regulator capable of a delivery pressure of 800 psig (5440 kPa) with a ¼ inch NPT male outlet should be suitable. In locations where imperial fittings are unavailable, a local fittings supplier should be able to provide NPT-metric converters to fit metric regulators. The LIPEX™ Extruders are available in several sizes dictated by the internal volume capacity; 1.5 mL, 10 mL, 100 mL and 800 mL (Note, the 100 mL and 800 mL Extruder are only offered with the thermobarrel, see below).

Quick Connect (QC) Fittings are included in the design of the LIPEX™ Extruders because of their easy push-pull operation, eliminating the need for twisting, turning or wrench action. The stainless steel delivery tubing is rated at 1500 psig (10,200 kPa) by the manufacturers. The relief valve is set to release at pressures in excess of 800 psig (5440 kPa), allowing for a large safety margin.

The 1.5 mL and 10 mL LIPEX™ Extruders are offered with the optional Thermobarrel. The Thermobarrel allows for precise thermostatted operation by allowing water from a circulating water bath to flow through a heat-jacketed reservoir surrounding the sample reservoir (circulating water bath not supplied). The ability to regulate the temperature of the lipid suspension is required when working with saturated phospholipids, which can only be extruded at temperatures above their main gel to liquid-crystalline phase transition temperature [for a review of phospholipid transition temperatures, see J.R. Silvius in Lipid-Protein Interactions Vol. 2 pp. 239-281 (Eds. P.C. Jost and O.H. Griffith) Wiley—Interscience, New York, 1982]. The thermobarrel extruder is not designed to heat or cool a product, but to maintain the temperature of the product. Therefore, the product being added to the thermobarrel should already be at the desired extrusion temperature. The Thermobarrel should be securely attached to a thermostatted circulator using only the high temperature tubing and screw clamps . A maximum operating temperature of 80°C is recommended.

The following manual describes the general assembly and operation of the LIPEX™ Extruder with our standard testing procedure. Please read this manual closely to ensure you get the maximum benefit from your newly purchased LIPEX™ Extruder.

2 Safety Considerations

- **Never operate your Extruder without use of the safety lanyard. Inspect the lanyard often for signs of wear, and replace as necessary.**
- **Do not adjust the pressure relief valve. If you require higher pressures, or the valve is not sealing properly, contact NLI for servicing.**
- **Inspect all O-rings frequently for signs of wear, and replace when necessary. Use of damaged or worn O-rings can result in a sudden rupture in the O-ring during operation, which can lead to a sudden depressurization of the Extruder.**
- **Rupture of an o-ring could result in expulsion of the liquid within the extruder under high pressure. It is therefore recommended that operators wear appropriate protective apparel and that the extruder be used within a fume hood and behind a safety shield.**
- **Care should be taken when handling LIPEX™ Extruders equipped with the thermobarrel as the thermobarrel may be hot and cause burns.**
- **Care should be taken when lifting and moving the larger LIPEX™ Thermobarrel Extruders to avoid physical injury.**

3 Parts List

The following describes the general parts supplied with a LIPEX™ 10 mL Thermobarrel Extruder. The only difference between the various sizes of LIPEX™ Extruders offered is the overall size and the number of wing-nuts and flat washers supplied to secure the top of the Extruder.

Assembly Number	Part Name	Quantity	
1	Tie Rod Base	1	
2	Filter support base with sample outlet tube	1	
3	Barrel or Thermobarrel (Thermobarrel is shown. It is differentiated from the barrel by the additional ¼" hose barb adaptors for connection to a thermostatted circulator)	1	
4	Large O-ring	2	
5	Small O-ring	1	
6	Stainless steel support mesh	1	

Assembly Number	Part Name	Quantity	
7	Stainless steel support disc	1	
8	Extruder Top	1	
9	Flat washer	3	
10	Wing nuts	3	

For the 100 mL LIPEX™ Extruder a total of 4 flat washers and wing-nuts are supplied
For the 800 mL LIPEX™ Extruder a total of 6 flat washers and wing-nuts are supplied

ADDITIONAL REQUIRED ITEMS

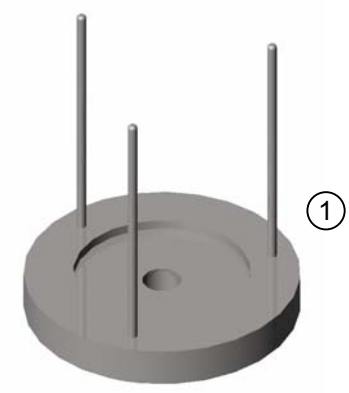
The following items are required for the proper operation of the LIPEX™ Extruder or LIPEX™ Thermobarrel Extruder. These items are not supplied with the LIPEX™ Extruder or Thermobarrel Extruder, but are available through Northern Lipids Inc. unless otherwise stated (see Appendix 1).

- Polycarbonate filters
- Polyester drain disks
- Nitrogen regulator
- Nitrogen gas cylinder (not available)
- Thermostatted circulator (not available and only required for the LIPEX™ Thermobarrel Extruders)

4 General Assembly Procedure

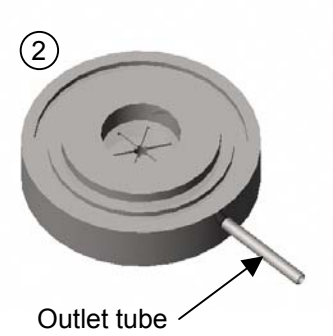
The following is a step-by-step assembly procedure for a 10 mL LIPEX™ Thermobarrel Extruder. The assembly procedure outlined is relevant to all available LIPEX™ Extruders. Please refer to the Parts List for the assembly number.

Step 1



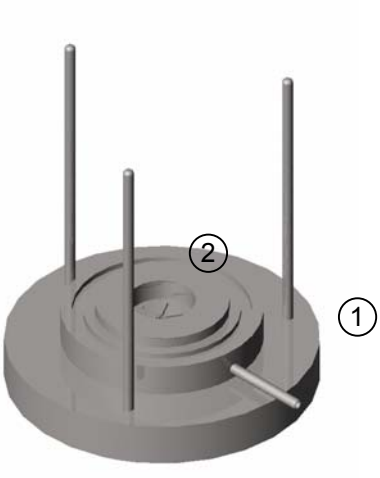
Place the Tie Rod base [1] on a suitable working surface. The working area should be clear of any unnecessary equipment.

Step 2



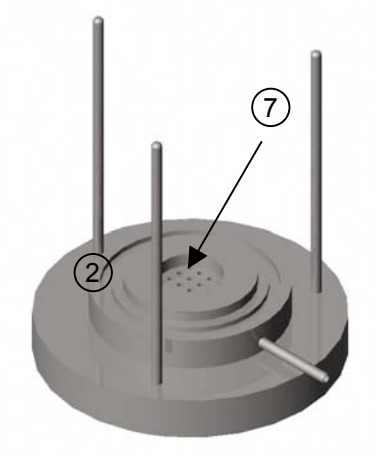
The LIPEX™ Extruders are supplied with a piece of Tygon tubing on the end of the outlet tube. Replace the tubing with an equivalent type of tubing if it appears damaged or worn. Ensure that the tubing is on securely and that approximately 3-4 mm of the tubing is over the outlet tube of the filter support base

Step 3



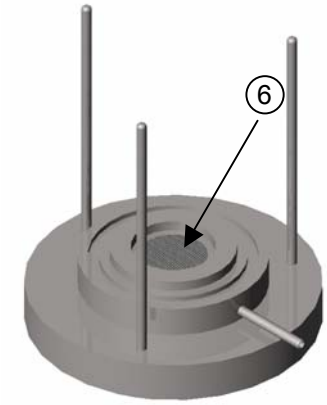
Place the filter support base [2] into the center cavity of Tie Rod base [1].

Step 4



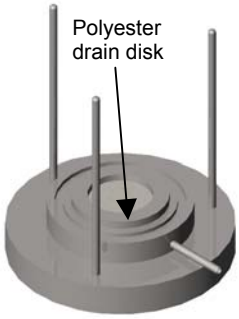
Place the stainless steel support disc [7] into the center cavity of the filter support base [2]

Step 5

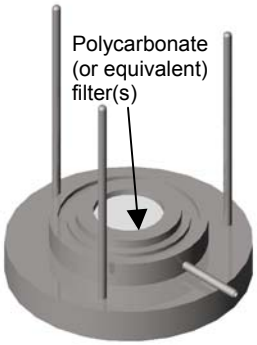


Place the supplied stainless steel mesh [6] on top of the stainless steel support disc [7].

Step 6

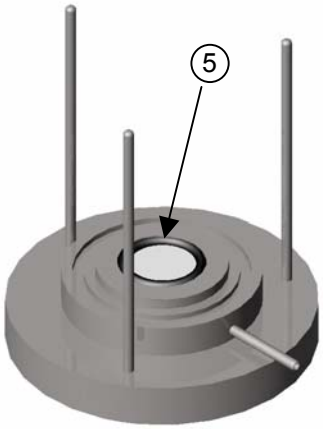


Add a few drops of distilled water or buffer to the stainless steel support mesh [6] to wet the support mesh. This aids in the placement of the polyester drain disk and polycarbonate (or equivalent) filter(s). Carefully place a single polyester drain disk onto the wetted stainless steel mesh [6]. Follow this by adding the required number of polycarbonate (or equivalent) filters onto the polyester drain disk. Ensure that the filters are well seated and have no creases or folds and that they are well centered such that a good seal will result.



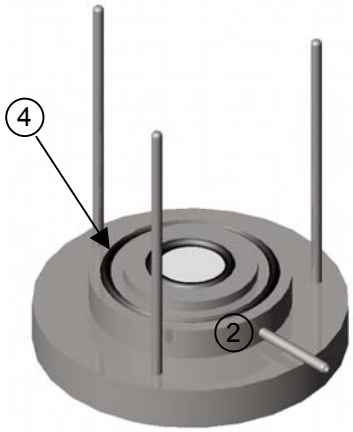
Note if multiple filters are used, the filters should be added individually and wetted in between each addition to help with placement of the filters. The polycarbonate filters that are available through NLI have a “shiny” side and a “blunt” side. Ensure that the filters are placed on the drain disc with the “shiny” side facing up. Also, each polycarbonate filter is packed in between two blue paper sheets. The blue paper sheets should be removed and discarded from each polycarbonate filter.

Step 6



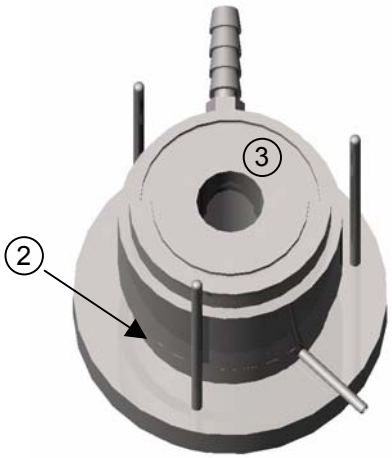
Carefully place the small O-ring [5] on top of the polycarbonate filters without disrupting the place of the filters or tearing the filters as this will result in improper size reduction of the liposomes.

Step 7



Place one of the supplied large O-rings [4] into the groove in the filter support base [2].

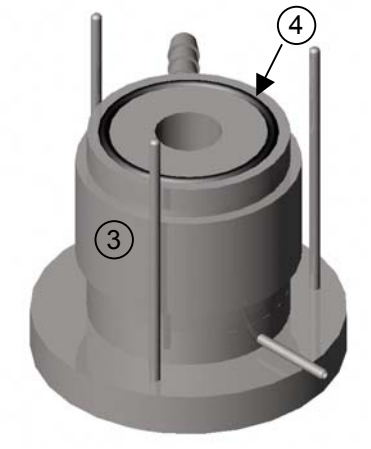
Step 8



Place the thermobarrel [3] squarely onto the filter support base [2] without disrupting the placement of the O-rings and filters in the filter support base. The side of the thermobarrel with the groove for the second large O-ring should be facing upward.

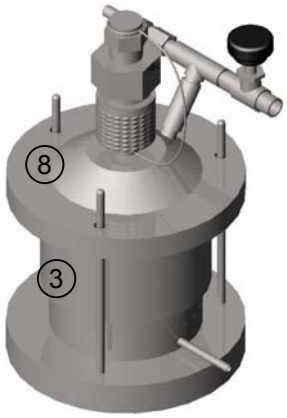
Note the rubber hose barb adaptors located on the side of the thermobarrel should be facing the opposite direction of the outlet tube of the filter support base to avoid any interference of the two.

Step 9



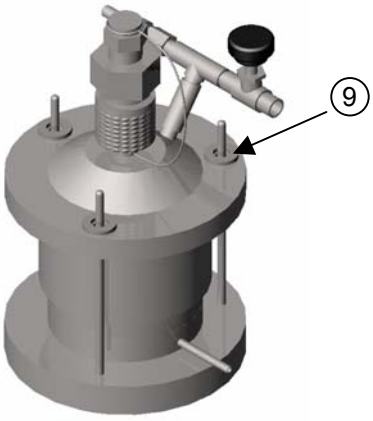
Place the second supplied large O-ring [4] into the groove of the top of the thermobarrel [3]

Step 10



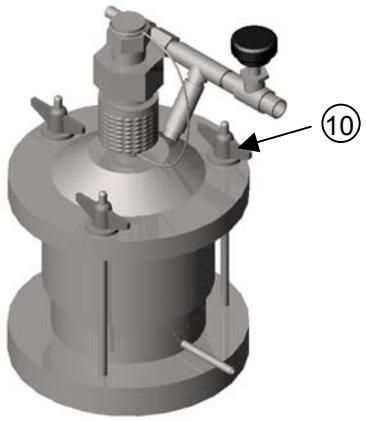
Carefully place the Extruder top [8] onto the thermobarrel [3]. The Extruder top should be positioned onto the rest of the Extruder so that the male gas quick connect (see figure 1) is pointing away from the outlet tube of the filter base support. This will make sample collection easier.

Step 11



Place the supplied flat washers [9] onto each of the protruding tie rods.

Step 12



Place each of the supplied wing-nuts [10] onto each of the protruding tie rods.

Tighten each wing-nut in an alternating fashion to ensure that the Extruder top is squarely secured onto the rest of the Extruder. The wing-nuts should be finger tightened.

Step 13

Couple the assembled 10 mL LIPEX™ Extruder to the nitrogen gas line (which is connected to the regulator (see figure 3) on the nitrogen cylinder) by attaching the female QC adaptor on the nitrogen gas line (see figure 2) to the male QC adaptor located on the Extruder Top (see figure 1). The female and male QC adaptors should "click" together.

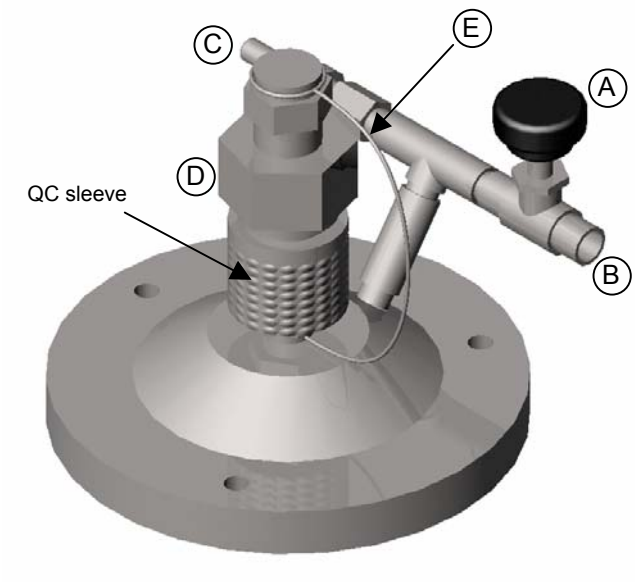


Figure 1 – Part designation of the Extruder Top

A – Pressure relief valve
B – Pressure relief port
C – Male QC adaptor for nitrogen gas
D – QC inlet cap for sample port
E – Safety lanyard

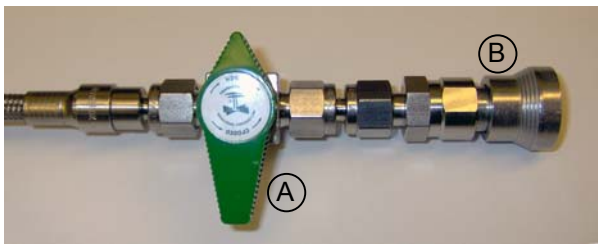


Figure 2 – Female QC adaptor portion of high-pressure nitrogen gas line

A – Green pressure control valve
B – Female QC adaptor
The valve is shown in the closed position. To open the valve, turn the valve so that the valve is parallel to the high-pressure nitrogen line.

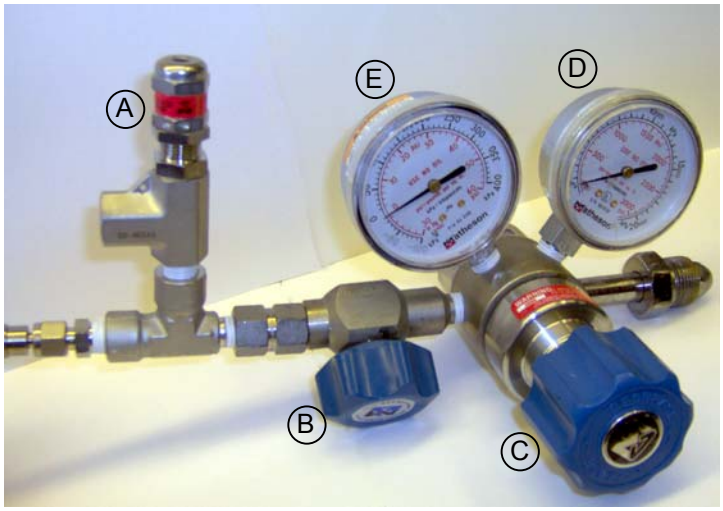


Figure 3 – Pressure regulator and pressure relief safety valve on the high-pressure nitrogen gas line.

A – Pressure relief safety valve (set to 800 psigg)
B – Needle valve
C – Main regulator control valve
D – Main tank pressure gauge
E – Delivery pressure gauge

Note – the pressure regulator shown is not included with the LIPEX™ Extruder, but is available through Northern Lipids Inc. (see Appendix 1).
Turning either the needle valve or main regulator control valve clockwise opens the valve while turning counterclockwise closes the valves

5 General Operating Procedure

The following procedure is a step-by-step description of the general operation of a 10 mL LIPEX™ Thermobarrel Extruder. The general steps are valid for all LIPEX™ Extruders. The preparation of egg phosphatidylcholine (EPC) liposomes at 50 mg/mL, which is used in our standard testing procedure, will be used to help describe the general extrusion process.

1. Prepare multilamellar vesicles (MLVs) consisting of EPC at 50 mg/mL

   Weigh out 250 mg of EPC and hydrate in 5 mL of 0.9% NaCl (0.9 g NaCl dissolved in 100 mL de-ionized water) to make a final lipid concentration of 50 mg/mL.

2. Assemble the 10 mL LIPEX™ Thermobarrel Extruder as described in section 3 of this operating manual with two stacked 100 nm polycarbonate filters (a start-up pack of 100 filters (100nm) is included in the extruder unit (see Appendix 1 or contact a NLI representative for other available polycarbonate filters).

3. For the extrusion of EPC vesicles, the thermobarrel does not have to be connected to a thermostatted circulator. However, when extruding lipid formulations that require temperature regulation attach the outlet tube from a thermostatted circulator to the lower hose barb adaptor on the thermobarrel and the return tube of the thermostatted circulator to the upper hose barb adaptor on the thermobarrel. Allow the thermobarrel to equilibrate to the required extrusion temperature prior to proceeding to the next step. At higher temperatures, greater than 50°C, the Extruder can be placed in a heated water bath so that the filter support base is below the water line. This improves the heat transfer of the thermobarrel, which can lead to improved extrusion.

4. Open the sample port by pulling upward on the sleeve of the QC inlet cap (see figure 3). Using a glass Pasteur pipette add the prepared EPC MLVs to the Extruder. Close the sample port by pushing the QC inlet cap down and over the sample port until there is a “click”.

   NOTE: After prolonged use the QC cap can become difficult to put on or take off. If this happens, simply wet the interior of the adaptor with distilled water or buffer. Grease should not be used as it may come into product contact.

5. Verify that the green pressure control valve (see figure 2), the main regulator control valve (see figure 3), and the pressure relief valve on the Extruder top (see figure 1) are in the closed position. Open the valve located on the nitrogen tank. The main tank pressure gauge on the regulator (see figure 3) should now register the pressure of the nitrogen tank in use. The delivery pressure gauge (see figure 3) on the pressure regulator should read zero. The pressure in the tank should be at least 1500 psig to ensure that there is enough nitrogen to complete the extrusion.

6. Set the delivery pressure by turning the dial on the nitrogen regulator clockwise until a reading between 200 and 300 psig (our suggested initial extrusion pressure) is observed. Fully open both the needle valve on the pressure regulator (see figure 3) and then the green pressure control valve (see figure 2). Once both of these valves are open, the lipid should start to extrude. If a sufficient rate of product flow is achieved, close the green pressure control valve (see figure 2); the remaining pressure in the Extruder is usually sufficient to expel the remaining lipid in the Extruder. If the lipid does not extrude or the flow rate is low, re-open the green pressure control valve and increase the nitrogen pressure by turning the nitrogen regulator dial counterclockwise until a sufficient product flow or a maximum of 800 psig of pressure is reached. Once product flow has started, close the green pressure control valve.

   NOTE: Do not attempt to remove the QC cap while the unit is pressurized! The cap is designed such that it is self-locking when under pressure. However, under moderate pressure it is possible to force the cap off.

   NOTE: As described above, it may be necessary to adjust the nitrogen pressure at the regulator so that the lipid is extruded at a reasonable rate. The pressure required to extrude at a moderate rate is dependent upon the filter pore size, lipid composition and concentration, buffer composition, and operating temperature. For this reason the working pressure should be determined for each sample and the regulator should not be preset to a particular pressure. Typically, to extrude egg phosphatidylcholine MLVs (50 mg/mL) through a 100 nm pore size filter requires an operating pressure of about 250 psig (1700 kPa). If the lipid sample is not easily extruded at the maximum operating pressure (800 psig, 5440 kPa) please refer to the Frequently Asked Questions in section 7 of this manual. To increase the extrusion pressure, slowly turn the main regulator control valve clockwise to open the valve. The reading on the delivery pressure gauge should slowly respond accordingly.

7. After the first extrusion pass is complete, close the green pressure control valve and depressurize the Extruder by opening the pressure relief valve on the Extruder top (see figure 1). Once the Extruder is depressurized close the pressure relief valve on the Extruder top. Refill the Extruder with the freshly extruded EPC liposomes and repeat the extrusion process for a total of 10 passes to ensure the final extruded product is homogeneous.

8. Upon completion of the tenth and final pass, close the valve on the nitrogen tank and open the pressure relief valve on the Extruder Top and the green pressure control valve to depressurize the Extruder and any remaining pressure in the high-pressure nitrogen gas line. Uncouple the extruder from the high-pressure nitrogen gas line once depressurization is complete by pushing forward on the female quick connect coupling (part B in Figure 2). Close the needle valve and the main regulator control valve. Proceed to cleaning extruder (see section 6).

   NOTE: Buffer can be passed through the Extruder (following the steps described above) prior to extruding the product to ensure that the Extruder was properly assembled and that there are no leaks in the system.

6 General Cleaning Procedure

The cleaning procedures described in this manual are only for the general cleaning of the LIPEX™ Extruder. It may be necessary to generate specific cleaning protocols for the particular compounds used with the Extruder.

1. The Extruder should be cleaned immediately after use. Disassemble the Extruder to expose all components of the Extruder.

2. We recommend cleaning all parts of the Extruder with a mild phosphate free cleaning solution. Use a brush for hard to reach places. When cleaning the base, use warm soapy water and be sure to flush the inside of the base with copious amounts of tap water.

3. Rinse each part extensively with tap water followed by several distilled water rinses.

4. Rinse all the stainless steel parts with 70% ethanol and wipe dry with a soft tissue. Do not expose the O-rings (including the O-ring in the QC inlet cap for the sample port) to an organic solvent such as 70% ethanol.

5. Special care should be taken to rinse the Extruder top and the QC inlet cap.

7 Frequently Asked Questions

Q: The Extruder seems to be leaking either gas or sample. How do I fix this?

A: If the Extruder leaks gas, or sample, re-assemble making sure that all the O-rings are mounted squarely. Test for leaks by placing a sheet of plastic over the filter support and then pressurizing the unit to 500 psig (3400 kPa) under water. The gas bubbles will indicate where the leak is. If it is from a fitting, unscrew and replace the Teflon tape around the threads. If an O-ring is leaking, replace it.

Q: My sample will not pass through the filters at all or takes several minutes to do so.

A: There could be several reasons for this problem:

a) The pore size of the filter is too small. Extrusion of MLV's through filters with a pore size of ≤ 100 nm may be slow even at 800 psig (5440 kPa). This will be dependent on many factors including lipid composition, phase transition temperature, lipid concentration, etc. Often extrusion may proceed more readily if a series of filters are used. For example, begin by sizing using 200 nm, followed by 100 nm and finally size to 80 nm filters.

b) The lipid is in the gel state at the operating temperature. Consult standard reference tables of phospholipid phase transition temperatures, e.g. J.R. Silvius in Lipid-Protein Interactions Vol. 2 pp. 239-281 (Eds. P.C. Jost and O.H. Griffith) Wiley—Interscience, N.Y. 1982.

c) The filters have become blocked by particulate material in the sample. If the buffer is the source of this material then prefiltration of the buffer alone, or extrusion through a series of filters as described in a) should solve this problem. More commonly, blame lies with the lipid sample itself. For example, during isolation using alumina or silica chromatography, small amounts of column "fines" are often eluted with the purified lipid. These can readily block the filters and should be removed by passing the lipid solution in organic solvent through Whatman filter papers using an all-glass filter funnel. In our experience, similar particulate matter can be present in some commercial preparations and we recommend purchasing NLI lipids: contact 1 888 654-7437, or email us at info@northernlipids.com

d) The lipid components in a mixture are not homogeneously distributed. Perhaps the most common example of this problem concerns mixtures of phosphatidylcholine and cholesterol. In our experience, when thin films of these two lipids are prepared by rotary evaporation from chloroform:methanol solutions, cholesterol can precipitate prior to deposition of the phospholipid. Following hydration of the film, extrusion of the sample is difficult due to blockage of the filters by these cholesterol microcrystals. For this and other lipid mixtures we therefore recommend lyophilization from solutions of tert-butyl alcohol or benzene: methanol (95:5 v/v).

Q: Why has the QC inlet Cap become stiff and difficult to use? What can be done to improve this?

A: With extended use the Quick-Connect cap can become stiff and difficult to use. This problem can be avoided by ensuring that the internal O-ring of this cap is kept moist. Do not grease the internal O-ring of the QC cap as this may come into product contact. Do not wash with organic solvent and do not remove the internal O-ring. After time the internal O-ring may wear down – replacement O-rings for this part can be obtained from Northern Lipids – use a spatula to tease out the old one and insert the replacement.

Q: Why can't I couple the Extruder to the pressure line?

A: The female QC portion of the high-pressure nitrogen gas line (see figure 2) automatically cuts off the nitrogen gas supply upon separation from the stem. As a result it is possible to pressurize the line up to

the QC whilst the Extruder is disconnected. The maximum backpressure safely allowed for the QC is 250 psigg (1700 kPa). At higher backpressures you will find it impossible to couple the Extruder to the pressure line. If, by accident, the line is pressurized to greater than 250 psig (1700 kPa) whilst the Extruder is uncoupled, release the pressure by first shutting off the nitrogen gas tank and slowly unscrewing the line out of the regulator.

Q: Why is the Thermobarrel not heating up?

A: Ensure the tubing from Thermobarrel to thermostatted circulator is properly connected and that there are no kinks in the tubing that would restrict the flow of water from the thermostatted circulator to the Thermobarrel.

Selected References

- Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) *Production of large unilamellar vesicles by a rapid extrusion procedure: Characterization of size distribution, trapped and ability to maintain a membrane potential.* Biochimica et Biophysica Acta 812, 55-65.
- Madden, L.D. and Cullis, P.R. (1985) *Preparation of reconstituted cytochrome oxidase vesicles with defined transmembrane protein orientations employing a cytochrome c affinity column.* Biochimica et Biophysica Acta 808, 219-224
- Mayer, L.D., Hope, M.J., Cullis, P.R. and Janoff, A.S. (1985) *Solute distribution and trapping efficiencies observed in freeze-thawed multilamellar vesicles*. Biochimica et Biophysica 817, 193-196.
- Hope, M.J., Bally, M.B., Mayer, L.D., Janoff, A.S. and Cullis, P.R. (1986) *Generation of multilamellar and unilamellar phospholipid vesicles.* Chemistry and Physics of Lipids 40, 89-108.
- Mayer, L.D., Hope, M.J. and Cullis, P.R. (1986) *Vesicles of various sizes produced by a rapid extrusion procedure.* Biochimica et Biophysica Acta 858, 161-168.
- Mayer, L.D., Bally, M.B., Hope, M.J. and Cullis, P.R. (1986) *Techniques for encapsulating bioactive agents into liposomes.* Chemistry and Physics of Lipids 40, 333-346.
- Bally, M.B., Nayar, R., Masin, D., Cullis, P.R. and Mayer, l.D. (1990) *Studies on the myelosuppressive activity of doxorubicin entrapped in liposomes.* Cancer Chemotherapy & Pharmacology 27, 13-19.
- Harrigan, P.R., Madden, T.D. and Cullis, P.R. (1990) *Protection of liposomes during dehydration or freezing.* Chemistry and Physics of Lipids 52, 139-149.
- Loughrey, H.C., Wong, K.F., Choi, L.S., Cullis, PO.R. and Bally, M.B. (1990) *Protein-liposome conjucates with defined size distributions.* Biochimica et Biophysica Acta 1028, 73-81.
- Allen, T.M., Austin, G.A., Chonn, A., Lin, L. and Lee, K.C. (1991) *Uptake of liposomes by cultured mouse bone marrow macrophages: influence of liposome composition and size.* Biochimica et Biophysica Acta 10612, 56-64.
- Eastman, S.J., Hope, M.J. and Cullis, P.R. (1991) *Transbilayer transport of phosphatidic acid in response to transmembrane pH gradients*. Biochemistry 30, 1740-1745.
- Konopka, K., Stamatatos, L., Larsen, C.E., Davis, B.R. and Duzgunes N. (1991) *Enhancement of human immunodeficiency virus type 1 infection by cationic liposomes: the role of CD4, serum and liposome-cell interactions*. Journal of General Virology 72, 2685-2696.
- Rafalski, M., Ortiz, A., Rockwell, A., Van Ginkel, L.C., Lear, J.D., Degrado, W.F. and Wilschut, J. (1991) *Membrane fusion activity of the influenza virus hemagglutinin: interaction of HA2 N-terminal peptides with phospholipid vesicles*. Biochemistry 30, 10211-10220.
- Allen, T.M., Mehra, T., Hansen, C. and Chin, Y.C. (1992) *Stealth liposomes: an improved sustained release system for 1-beta-d-arabinofuranosylcytosine*. Cancer Research 52, 2431-2439.
- Bakker-Woudenberg, I.A., Lokerse, A.F., Ten Kate, M.T. and Storm, G. (1992) *Enhanced localization of liposomes with prolonged blood circulation time in infected lung tissue*. Biochimica et Biophysica Acta 1138, 318-326.

- Chonn, A., Semple, S.C. and Cullis, P.R. (12992) *Association of blood proteins with large unilamellar liposomes in-vivo: Relation to circulation lifetimes*. Journal of Biological Chemistry 267, 18759-18765.
- Eastman, S.J., Hope, M.J., Wong, K.F. and Cullis, P.R. (1992) *Influence of phospholipid asymmetry on fusion between large unilamellar vesicles*. Biochemistry 31, 4262-4268.
- Gabizon, A.A. (1992) *Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes*. Cancer Research 52, 891-896.
- Suntres, Z.E., Hepworth, S.R. and Shek, P.N. (1992) *Protective effect of liposome associated alpha-tocopherol against paraquat-induced acute lung toxicity*. Biochemical Pharmacology 44, 1811-1818.
- Huang, S.K., Martin, F.J., Jay, G., Vogel, J., Papahadjopoulos, D. and Friend, D.s. (1993) *Extravasation and transcytosis of liposomes in Kaposi's sarcoma-like dermal lesions of transgenic mice bearing the HIV tat gene*. American Jouyrnal of Pathology 143, 10-14.
- Mui, B.L., Cullis, P.R., Evans, E.A. and Madden, T.D. (1993) *Osmotic properties of large unilamellar vesicles prepared by extrusion*. Biophysica Journal 64, 443-453.
- Zhu, N., Liggitt, D., Liu, Y. and Debs, R. (1993) *Systemic gene expression after intravenous DNA delivery into adult mice.* Science 261, 209-211.
- Litzinger, D.C., Buiting, A.M.J., Van Rooijen, N. and Huang, L. (1994) *Effect of liposome size on the circulation time and intraorgan distribution of amphipathic poly (ethylene glycol) – containing liposomes.* Biochimica et Biophysica Acta 1190, 99-107.

Contact and General Information

LIPEX™ is a division of Northern Lipids Inc. and has been designing and supplying extruders for over the past 15 years. We offer a wide range of LIPEX™ product to meet all your extrusion needs. From the initial research and development to the commercial manufacturing we will have an extrusion system that will meet all of your needs. We offer our standard Extruder and Filter Holders to customized extrusion systems. For further information please feel free to contact us.

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Appendix 1

The following items for the LIPEX™ Extruders are available through NLI. Please contact us for current price and availability.

Catalogue Number	Item Description
	Complete Extruders
B.002	1.5 mL LIPEX™ Extruder
T.002	1.5 mL LIPEX™ Thermobarrel Extruder
B.001	10 mL LIPEX™ Extruder
T.001	10 mL LIPEX™ Thermobarrel Extruder
T.100	100 mL LIPEX™ Thermobarrel Extruder
T.800	800 mL LIPEX™ Thermobarrel Extruder
	Thermobarrel or Barrel only
B.016	1.5 mL LIPEX™ Extruder
T.006	1.5 mL LIPEX™ Thermobarrel Extruder
B.015	10 mL LIPEX™ Extruder
T.005	10 mL LIPEX™ Thermobarrel Extruder
	Complete O-rings sets
B.010	1.5 mL LIPEX™ Extruder or Thermobarrel Extruder
B.009	10 mL LIPEX™ Extruder or Thermobarrel Extruder
T.107	100 mL LIPEX™ Thermobarrel Extruder
T.807	800 mL LIPEX™ Thermobarrel Extruder
	Filter Support Base
B.006	1.5 mL LIPEX™ Extruder or Thermobarrel Extruder
B.005	10 mL LIPEX™ Extruder or Thermobarrel Extruder
	Stainless steel support mesh
B.011	1.5 mL LIPEX™ Extruder or Thermobarrel Extruder
B.012	10 mL LIPEX™ Extruder or Thermobarrel Extruder
T.108	100 mL LIPEX™ Thermobarrel Extruder
T.808	800 mL LIPEX™ Thermobarrel Extruder
	Stainless steel support disc
B.014	1.5 mL LIPEX™ Extruder or Thermobarrel Extruder
B.013	10 mL LIPEX™ Extruder or Thermobarrel Extruder
T.109	100 mL LIPEX™ Thermobarrel Extruder
T.809	800 mL LIPEX™ Thermobarrel Extruder
	Accessories
X.001	High pressure nitrogen gas line
X.002	Nitrogen regulator
Polycarbonate filters and Drain disk for the 1.5 mL LIPEX™ Extruder or Thermobarrel Extruder	
87481	13 mm polyester drain disk
110403	13 mm polycarbonate filter (50 nm pore size)
110404	13 mm polycarbonate filter (80 nm pore size)
110405	13 mm polycarbonate filter (100 nm pore size)
110406	13 mm polycarbonate filter (200 nm pore size)

Catalogue Number	Item Description
Polycarbonate filters and Drain disk for the 10 mL LIPEX™ Extruder or Thermobarrel Extruder	
230600	25 mm polyester drain disk
110603	25 mm polycarbonate filter (50 nm pore size)
110604	25 mm polycarbonate filter (80 nm pore size)
110605	25 mm polycarbonate filter (100 nm pore size)
110606	25 mm polycarbonate filter (200 nm pore size)
Polycarbonate filters and Drain disk for the 100 mL LIPEX™ Thermobarrel Extruder	
231100	47 mm polyester drain disk
111103	47 mm polycarbonate filter (50 nm pore size)
111104	47 mm polycarbonate filter (80 nm pore size)
111105	47 mm polycarbonate filter (100 nm pore size)
111106	47 mm polycarbonate filter (200 nm pore size)
Polycarbonate filters and Drain disk for the 800 mL LIPEX™ Thermobarrel Extruder	
87490	90 mm polyester drain disk
111703	90 mm polycarbonate filter (50 nm pore size)
111704	90 mm polycarbonate filter (80 nm pore size)
111705	90 mm polycarbonate filter (100 nm pore size)
111706	90 mm polycarbonate filter (200 nm pore size)