Description and Operating Instructions for LiposoFast™

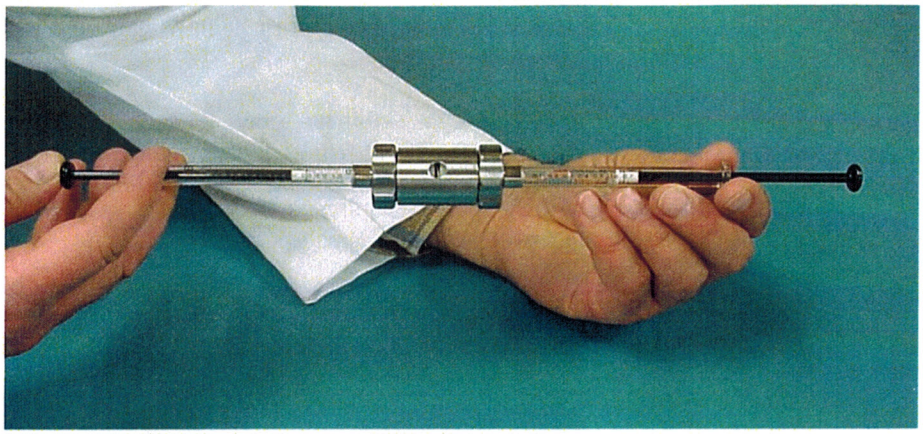


Figure 1: LiposoFast-Basic. Preparation of liposomes by extrusion through a membrane.

1. Principle of operation: A lipid emulsion is extruded repeatedly through a polycarbonate membrane. Depending on the formulation, 11 to 21 passes are sufficient to produce a liposome population of uniform size. The design of this instrument is based on MacDonald, R.C. et. Al. (1991) Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. Biochimica et Biophysica Acta 1061: 297-303.

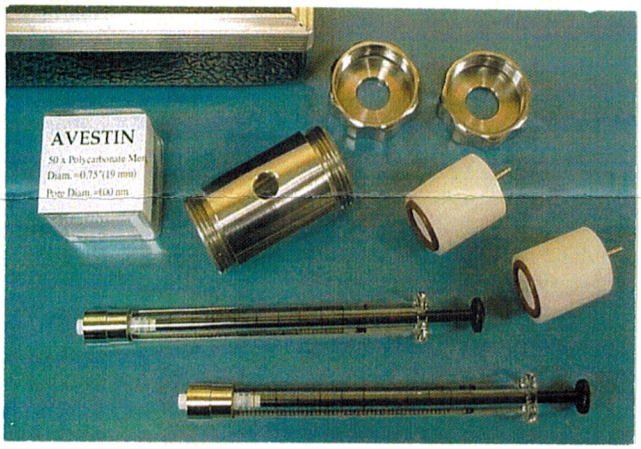


Figure 2: The LipsosFast-Basic consists of a stainless steel housing, membrane support, two 0.5mL syringes, and 50 polycarbonate membranes with 100nm pore diameter.

2. Capacity: The instrument's capacity is from 0.1 to 1.0mL, making it extremely useful for preparing large numbers of small samples for research.

3. Trial: LiposoFast products are available for a free trial. Please visit www.avestin.com for more information.

4. Stabilizer: The LiposoFast-Stabilizer was designed to facilitate repetitive use and extrusion of highly concentrated emulsions. The Stabilizer can accommodate both 0.5 and 1.0mL syringes.

5. Temperature control: The LiposoFast-Basic and Stabilizer can be immersed in a temperature controlled water bath.

6. Operating Instructions:

6.1 Clean the LiposoFast with alcohol prior to use. It can also be autoclaved.

6.2 Insert one of the two plastic units of the membrane support system into the steel housing with the "O"-ring side outward. Leave a few mm exposed at the top.

6.3 Lay one or two membranes over the "O"-ring (see Figures 3 and 4). (Note: Membranes are shiny, opaque circles enclosed between two protective paper discs).

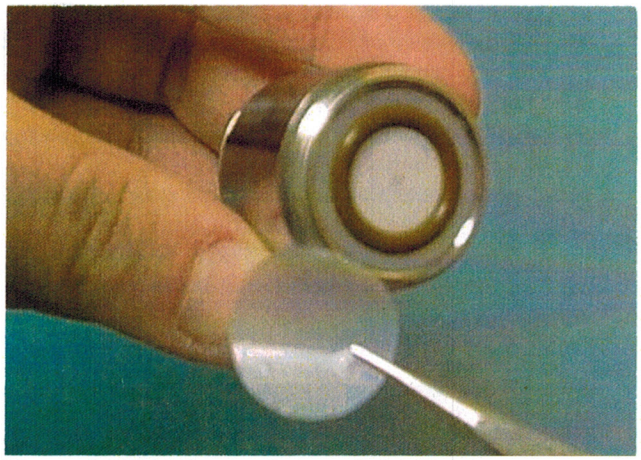


Figure 3: Place the membrane on the "O"-ring.

6.4 Place the other plastic unit of the membrane support system into the housing with the membrane sandwiched between two "O-rings. The support system should be positioned so that the membrane is centrally located when viewed through the inspection hole.

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Figure 4: You can view the membrane through the inspection hole.

6.5 Install and tighten end caps by hand. The membrane should be firmly pressed between two "O"-rings. The support system and membranes can be damaged if the end caps are over tightened.

6.6 Prepare multilamellar liposomes, first by dissolving phospholipids in an organic solvent and then by removing the solvent using rotary evaporation to produce a lipid film. Hydrate film with aqueous phase and shake by hand or with a mechanical shaker to produce multilamellar liposomes. Other reliable methods of preparing multilamellar liposomes can also be used.

6.7 Fill one syringe with multilamellar liposome preparation. Attach the filled syringe to the luer lock of one of the pieces of the membrane support by gently pressing the syringe straight on all the way until it stops. Screw the syringe about one quarter-turn clockwise to seal. Do not turn too much as the luer lock can be damaged. Attach an empty syringe to the other side. With a proper assembly, results are reproducible and dead space is minimized to only a few μL. Improper operation will damage the plastic luer lock. Metallic luer locks cannot be installed as they introduce the danger of contamination by metallic particles.

6.8 Pass the liposome emulsion back and forth through the membrane(s). Usually 11 to 21 passes are sufficient. (See Figure 5.)

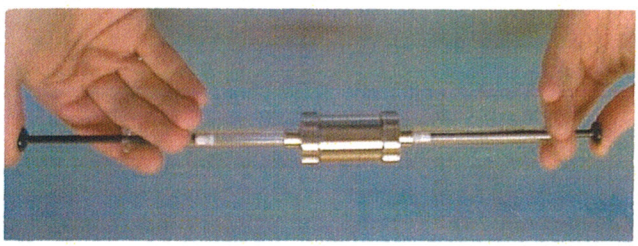


Figure 5: Push the sample by hand from one syringe to another across the membrane.

6.9 Remove the liposomes from the originally empty syringe to eliminate any unextruded vesicles. Be sure to unscrew the syringe about one quarter-turn counter-clockwise before pulling it off gently.

6.10 To use the Stabilizer, secure the base plate of the Stabilizer to a bench using a simple clamp. Insert the LiposoFast into the grooving of the Stabilizer. Close the lid of the Stabilizer and tighten the locks. Do not over tighten as the syringes can be damaged. (See Figure 6.)

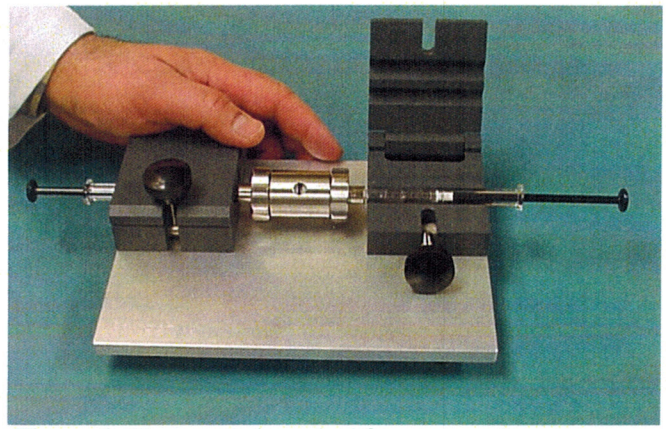


Figure 6: The sample is pushed from one syringe to another across the membrane while the LiposoFast-Basic is secured by the Stabilizer.

7. Components: The LiposoFast-Basic comes with a box of 50 polycarbonate membranes with a pore diameter of 100nm. Polycarbonate membranes with pore sizes of 50, 100, 200, 400, 800, 1000, and 5000nm are also available. The standard LiposoFast is supplied with two 0.5mL gas tight syringes. Also available are 0.25 and 1.0mL syringes.

8. Scaling Up: The LiposoFast LF-50 is a medium pressure extruder which uses compressed gas at pressures up to 600psi/41bar to pressurize the samples from 5mL to 50mL and force the starting materials through a polycarbonate membrane.

Avestin also manufacture high pressure homogenizers that are ideal for ultra-fine emulsions, homogeneous liposomes, bacteria and yeast cell rupture, etc. Standard homogenizers are designed for pressures up to 30000psi/2000bar, and volumes from 7mL batch to 1000L/h production.

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