Liposomes

Description and Operating Instructions for LiposoFast™

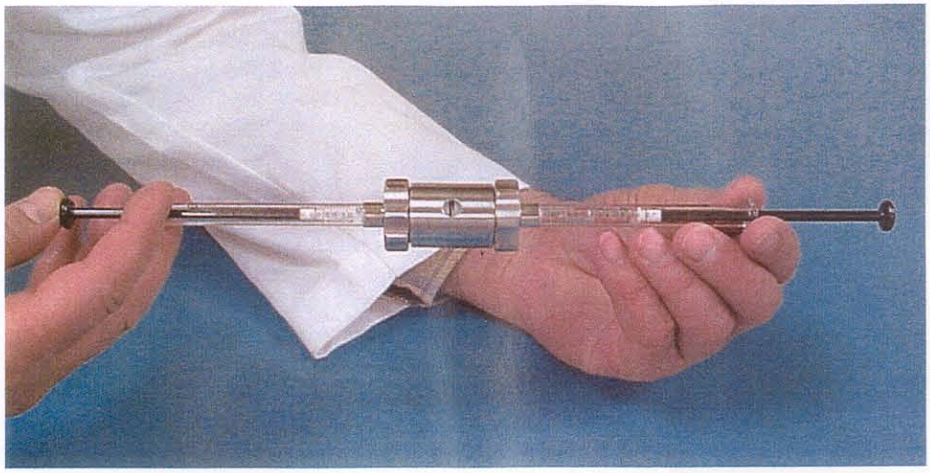


Figure 1: LiposoFast-Basic. Preparation of liposomes by extrusion through a membrane.

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 LiposoFast-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. Pneumatic-Actuator: The LiposoFast Pneumatic-Actuator uses a small amount of compressed air or gas for semi-automatic preparation of liposome samples. It is ideal for large numbers of reproducible samples.

6. Temperature control: The LiposoFast-Basic and Stabilizer can be immersed in a temperature-controlled water bath. The LiposoFast Pneumatic-Actuator can be immersed in a temperature-controlled water bath, using just enough water to cover the LiposoFast-Basic.

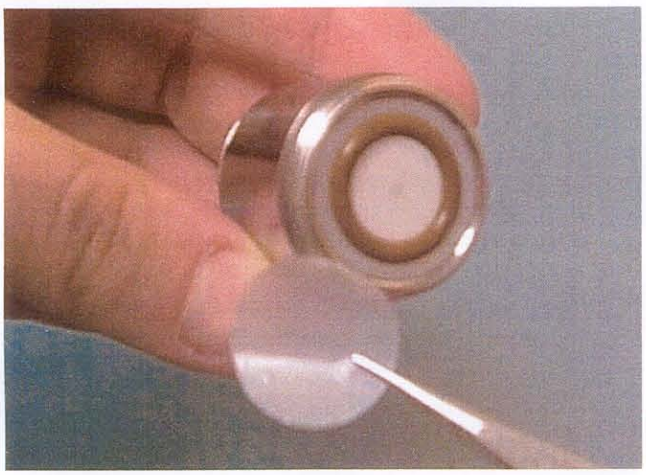
Avestin, Inc. 2450 Don Reid Drive, Ottawa Canada, K1H 1E1 Tel: +1-613-736-0019 Fax: +1-613-736-8086	Avestin Europe GmbH Weinheimer Str. 64b 68309 Mannheim, Germany Tel: +49-621-724-5980 Fax: +49-621-724-5813	**www.avestin.com** avestin@avestin.com

7. Operating instructions:

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

7.2 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.

7.3 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).



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

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

7.4 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.

7.5 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 overtightened.

7.6 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 multilammellar liposomes. Other reliable methods of preparing multilamellar liposomes can also be used.

7.7 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.

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

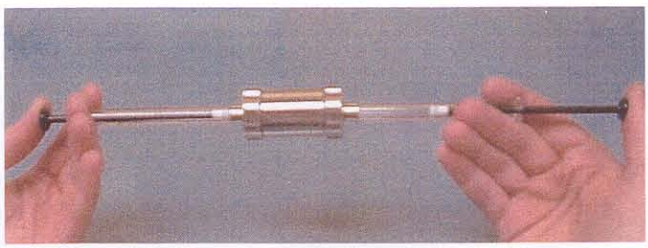


Figure 5: Push the sample by hand from one syringe to another across the membrane.

7.9 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.

7.10 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 overtighten as the syringes can be damaged. (See Figure 6).

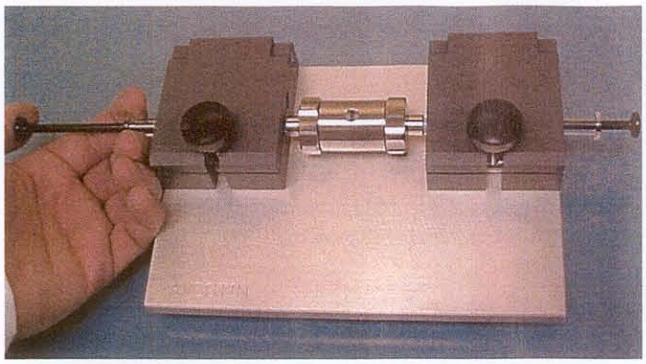


Figure 6: The sample is pushed from one syringe to another across the membrane while the LiposoFast-Basic is secured by the Stabilizer.

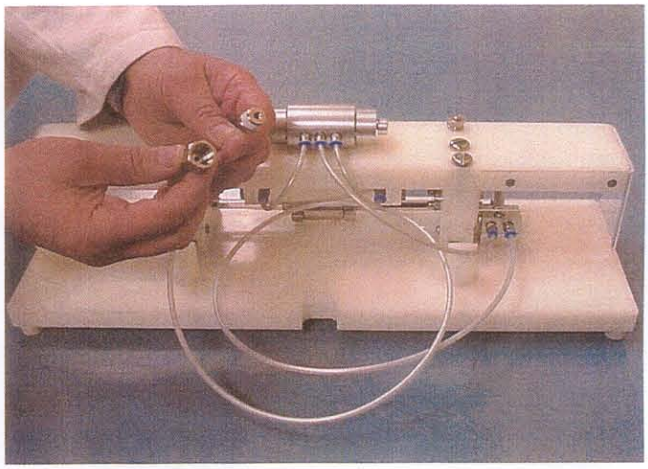


Figure 7: One meter of inlet tubing is provided with the LiposoFast Pneumatic-Actuator. This can be connected to your compressed air/gas system using the 1/4" male connector as shown. A 1/4" female connector is also supplied as an alternative.

8. Operation of the LiposoFast Pneumatic-Actuator

8.1 Take all necessary precautions to ensure safe operation of the LiposoFast Pneumatic-Actuator. Wear eye protection, gloves, and protective clothing at all times. Operate the instrument in a contained area when working with pathogenic, toxic, or corrosive materials.

8.2 Ensure that the instrument is clean and free of any debris that could interfere with operation. Do not attach the Pneumatic-Actuator to any compressed air/gas source with a pressure greater than 100psi / 7bar.

8.3 The Pneumatic-Actuator requires a source of compressed air or gas. Use any non-explosive and non-dangerous gas, such as nitrogen. Attach the air inlet tube to the air/gas source using either the male or female 1/4" fitting (both are provided with the instrument). The Pneumatic-Actuator requires a minimum of 30psi / 2bar to operate. The exact pressure necessary for extrusion will depend on the specific liposome formulation being prepared. Operation at too high a pressure will result in sample loss due to leaking. Do not operate the actuator above 100psi / 7bar. Try using the Pneumatic-Actuator while it is empty as well as with water in the syringes in order to become familiar with the instrument.

8.4 Assemble the LiposoFast-Basic as described in section 7, using 1.0mL syringes (provided with the Pneumatic-Actuator). 0.5mL syringes cannot be used, they are too small for proper operation.

8.5 Place the LiposoFast on the lower clamps of the Pneumatic-Actuator (See figures 8 and 9). Processing should always begin with the syringe containing the multilamellar liposome solution on the same side, preferably the right. This is to ensure process consistency among different users. It is important that the piston of the Pneumatic-Actuator be positioned to the same side as the full syringe. The plunger of the empty syringe must be fully inserted.

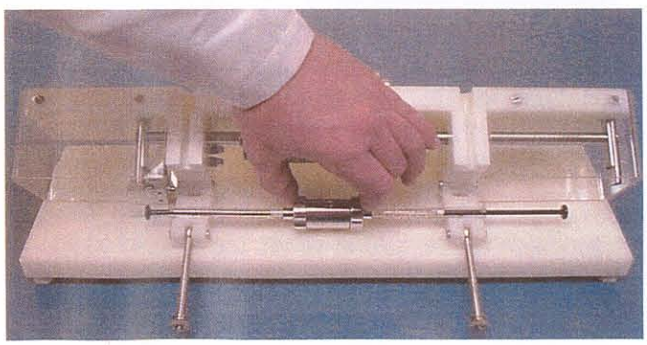


Figure 8: Place the LiposoFast-Basic on the lower clamps of the Pneumatic-Actuator. The support housing is suspended between the two clamps.

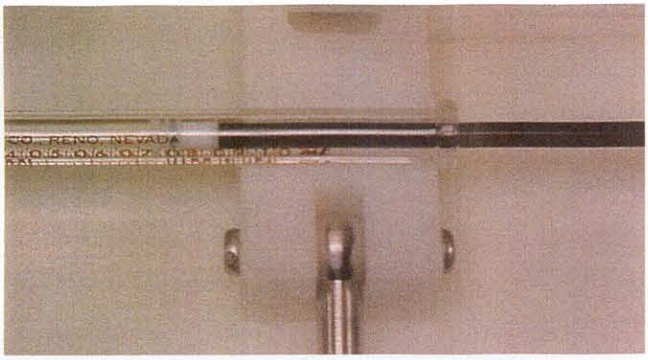


Figure 9: Proper syringe position when the LiposoFast-Basic is installed on the LiposoFast Pneumatic-Actuator.

8.6 Close the lid of the LiposoFast Pneumatic-Actuator. Be careful not to crush or bend the syringe plungers. Check that the clamps are properly closed. Tighten the nuts on top of the eyebolts to secure the lid. (See Figure 10). The safety switch will prevent air from entering the instrument if the lid is not closed completely. Do not tamper with the safety switch.

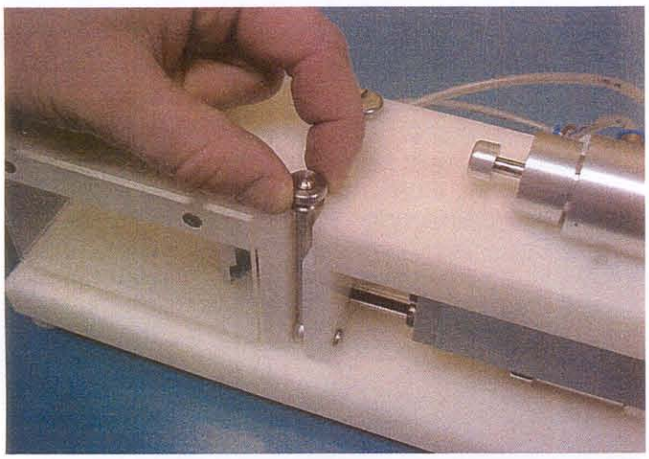


Figure 10: When the lid of the Pneumatic-Actuator is closed properly, the clamps are completely shut. Lift the eyebolts into place and tighten the nuts to secure the lid.

8.7 Pass the liposome emulsion back and forth through the membrane(s) using the push buttons on either side of the control valve. Usually, 11 to 21 passes are sufficient. (See Figure 11).

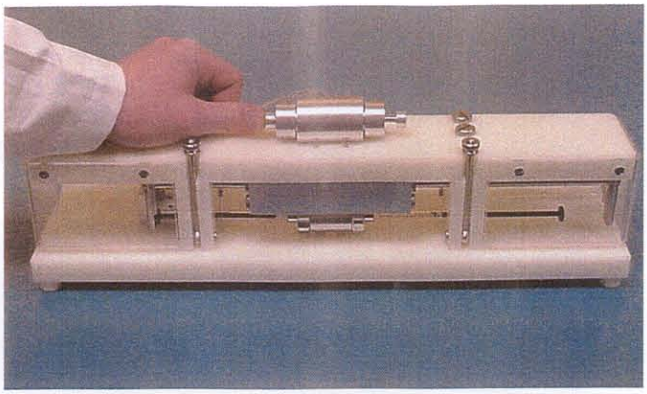


Figure 11: Operate the LiposoFast Pneumatic-Actuator using the push buttons on either side of the control valve.

8.8 Remove the liposomes from the originally empty syringe to ensure that no unextruded vesicles are contained in the final, unilamellar liposome solution. Be sure to unscrew the syringe about one quarter-turn counter-clockwise before pulling it off gently.

9. Components

9.1 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. 0.25 and 1.0mL syringes are also available.

9.2 The LiposoFast Pneumatic-Actuator comes with two 1.0mL gas tight syringes, 1.0m of 4mm inlet tubing and 1/4" male and female fittings (compatible with both NPT and ISO standard fittings) to connect to your compressed air or gas system.

10. Scaling Up: Avestin also manufactures 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.

Avestin, Inc. 2450 Don Reid Drive, Ottawa Canada, K1H 1E1 Tel: +1-613-736-0019 Fax: +1-613-736-8086	Avestin Europe GmbH Weinheimer Str. 64b 68309 Mannheim, Germany Tel: +49-621-724-5980 Fax: +49-621-724-5813	**www.avestin.com** avestin@avestin.com