USER GUIDE

FLUIGENT
SMART MICROFLUIDICS™

LIPOSOME PRODUCTION PACK

PRODUCT DESCRIPTION

P/N: O-MIX-LIPO-PCK

The following document presents all the steps to begin and end your liposome production experiment.

CONTENT

For additional information, contact us by email : contact@fluigent.com or consult our website : www.fluigent.com or

ALWAYS WEAR APPROPRIATE PERSONAL PROTECTIVE EQUIPEMENT

Safety glasses

Adequate Gloves

Lab Coat

OVERVIEW: MICROFLUIDIC LIPOSOME PRODUCTION USING A HERRINGBONE MIXER CHIP

Liposomes are spherical vesicles composed of phospholipid bilayers. They can encapsulate both hydophilic and hydrophobic active pharmaceutical ingredients (API), making them useful for drug delivery. The organization in vesicles and the encapsulation of molecules is due to lipid properties: hydrophobic tail and hydrophilic head. Hydrophobic tails cannot be in contact with the aqueous phase and consequently shape themselves in a vesicle.

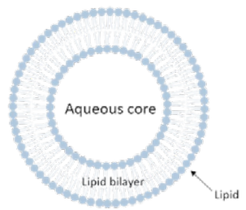


Figure 1: Simple liposome structure.

Microfluidics offers greater efficiency and precision compared to traditional methods such as thin-film hydration and extrusion. This method is based on a staggered herringbone mixer chip that generates a chaotic flow profile between the lipid and aqueous phases, characterized by a decreasing diffusion length between the streams. This process facilitates rapid mixing and enables lipids to assemble into liposomes. By tuning the lipid concentration, flow rate ratio, and total flow rate, the size and monodispersity of the liposome can be efficiently controlled. The Liposome Production Pack controls the flow ratios precisely using our Flow EZ flow controllers. This results in liposomes of uniform size.

Liposomes are created following the formluation below:

LIPOSOMES PRODUCTION	
Lipid Phase	Lipids in organic solvent (depending on the application) Here, absolute ethanol, lipids (40mg/mL), and Dimethyldioctadecylammmonium (DDAB - 10 mg/mL) were used.
Aqueous phase	Phosphate-buffered saline (PBS) (ph 7.2)
Particle Size Range*	40 nm to 150 nm
Polydispersity*	From 10% to 20%

* Note that the size and the polydispersity of the liposome depends on the chosen lipid formulation.

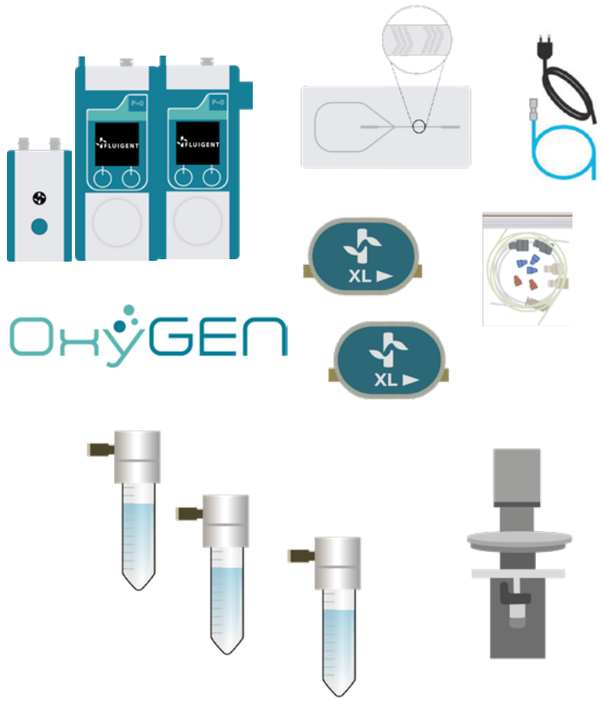
PACKAGE CONTENTS

2 * Flow EZ™ (7000 mbar)
1 * Link
2 * FLOW UNIT (XL)
2 * P-CAP (2* 15 mL)
1 * P-CAP (50 mL)
1 * Herringbone mixer chip
1 * Lineup supply kit
1 * Flow Unit XL tubing kit
1 * High flow rate kit
2 * P-CAP 15 mL kit
1 * P-CAP 50 mL kit

1 * Liposome tubing kit (3 caps 1/4-28, 3 connectors/plugs 1/4-28, 6 yellow-steel ring ferrules, tubing diameter (1/16 “OD 0.020“ ID) 1m, tubing cutter).

OxyGEN Software

1 * Digital High-speed Opto Microscope (optional)



PRESSURE CONTROLLERS

Two Fluigent Flow EZ’s (7 bar) are used in the setup. The use of pressure instead of syringe pumps to drive liquid flows provides a system that responds up to ten times faster. A LineUp Link module is provided to connect to the Oxygen Software for direct flow control.

Figure 2: Fluigent’s Flow EZ 7 bars.

FLOW SENSORS

The Flow Unit is a flow sensor that allows the flow rates to be directly controlled or measured. By combining a Flow Unit with the Flow EZ, users can switch from pressure control to flow rate control. Two Flow Units are used to monitor and control the flow rates of the aqueous and lipid phases.

Figure 3: Fluigent’s Flow Unit XL and low pressure union.

STAGGERED HERRINGBONE MIXER

The staggered herringbone mixer is designed with an inlet mixing section, and outlet. The inlet is a flow-focusing deisgn, which permits viewing and easy control of the aqueous and lipid phases before mixing. The mixing section is formed by 30 half-cycles including 6 herringbone structures each. Manufactured in glass, the chip can be cleaned and reused and is easily connected to the tubing with 1/4-28 fittings.

MICROFLUIDIC CHIP	
Herringbone Micromixer Chip	Little Things Factory - T29
Material	Borofloat®33
Dimension (L/W/H)	75 x 25 x 2 ± 0.3 mm
Mixer Volume	0.47 µL
Mixer Length	28.7 mm
Channel Depth	0.08 – 0.11 mm
Channel Width	0.1 – 0.5 mm
Pressure resistance mixer	15 bar

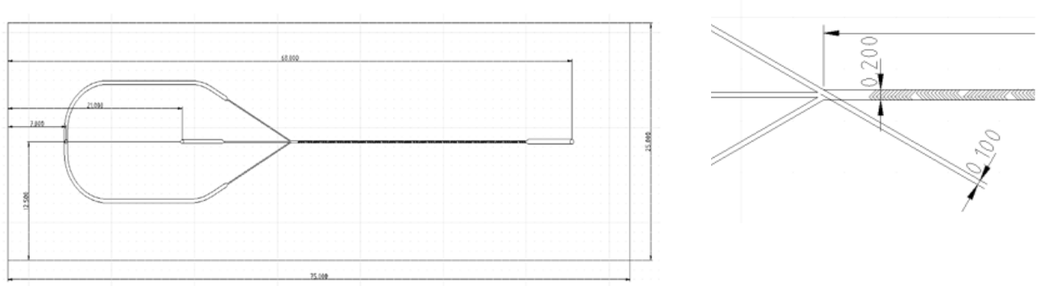


Figure 4: Characteristics of the herringbone mixer chip.

INITIAL PREPARATION

1. Connect the LineUp module to the two Flow EZ pressure controllers (7 bar). Connect the Flow EZ to the P-CAP fluid reservoirs. Depending on the phase's volume, fill the falcon reservoirs accordingly. For this application, the aqueous phase was placed in the 50 mL P-CAP reservoir and the solvent phase in the 15 mL P-CAP reservoir.

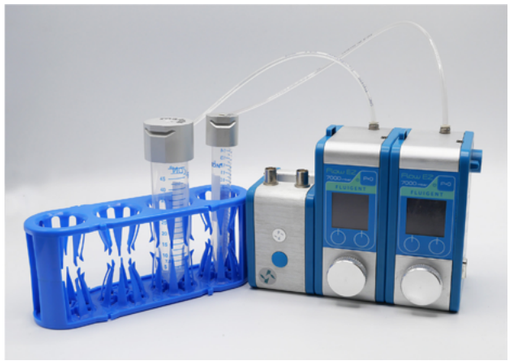


Figure 5: Connection of P-CAP to Flow EZ.

2. Connect the reservoirs (P-CAP) to the low pressure union and the Flow Unit following the image below.

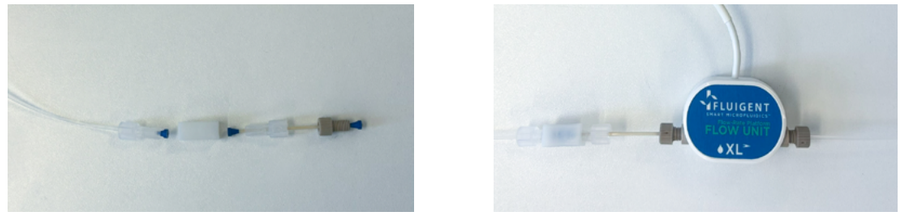


Figure 6: Flow. Unit connected to the low pressure union.

Be careful not to bend or damage the low pressure union throughout the experiments.

3. Connect the Link Module to Oxygen Software.

4. Calibrate each of the Flow Unit XLs according to their respective liquids. The principle of the calibration is to flow the phase through the Flow Unit. Then simultaneously record the flow rate given by the software and measure the weight of fluid you have collected over a defined time period. Knowing the density of the fluid, you can define the actual flow rate.

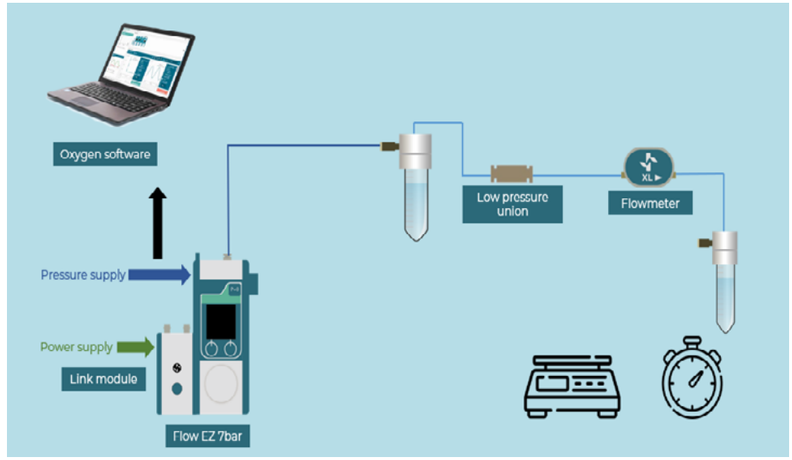
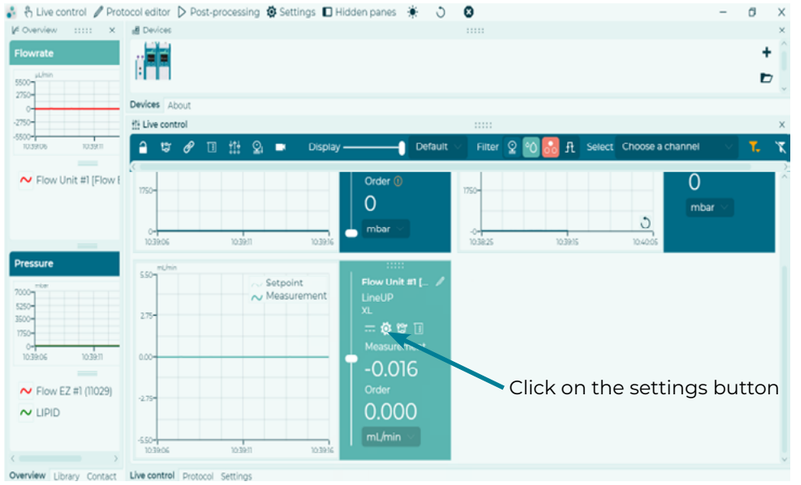


Figure 7: Set-up for Flow Unit calibration.

Make a table that contains the time for each measurement of the flow rate measured by the Flow Unit and the actual flow rate. A minimum of 3 measurements are recommanded for each flow rate. The resulting scale between the measured and actual flow rate can be added in Oxygen as described below.



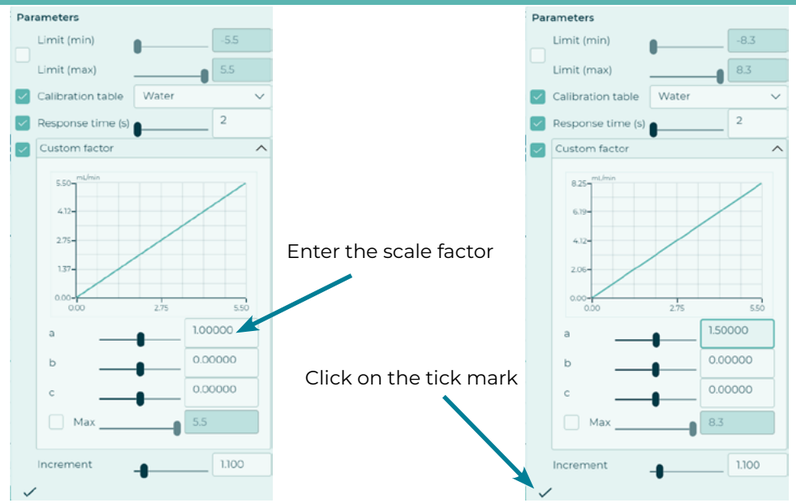


Figure 8: Procedure for adding scale factor in Oxygen.

Adding the scale factor ensures that the flow sensor reading is now accurate for the target fluid. For more details, please check the Flow Unit user manual.

5. Connect the herringbone mixer inlets to the Flow Units, using the fittings and yellow-steel ring ferrules.

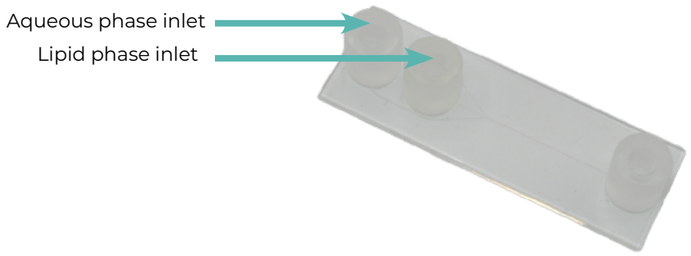


Figure 9: Inlets for aqueous and lipid phases.

6. Connect the exit of the herringbone mixer to a collection reservoir using a fitting and yellow-steel ring ferrules.

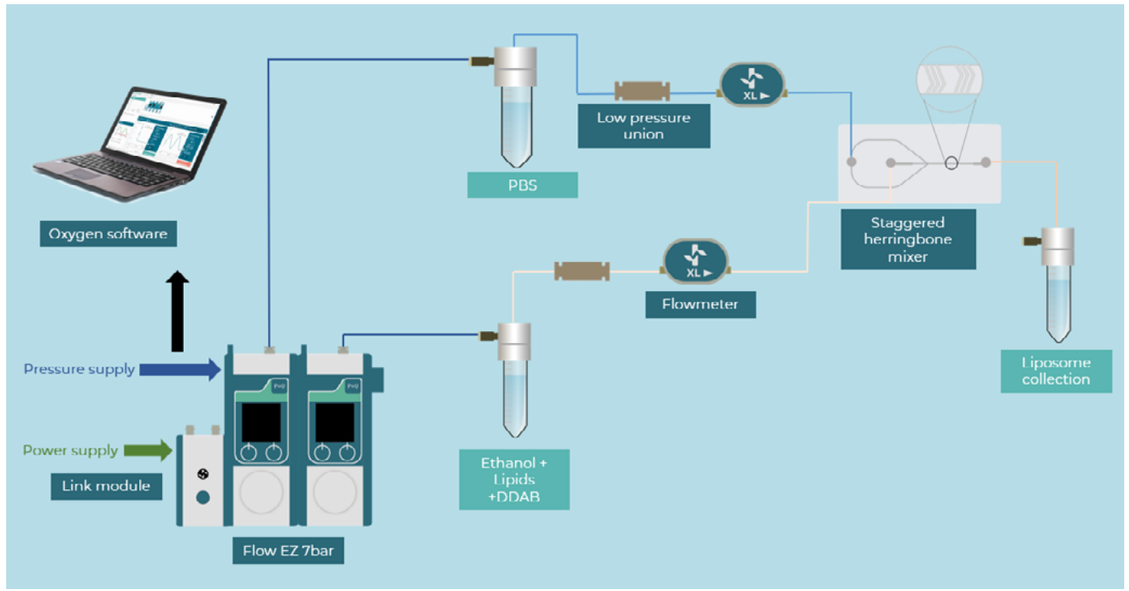


Figure 10: Set-up of liposome production pack.

LIPOSOME PRODUCTION PROTOCOL

We recommend observing the inside of the chip under a microscope. In the User Guide, the Opto Digital High-Speed Microscope was used alongside its software (Optoviewer).

We recommend filtering the solutions using the appropriate 0.2 µm filters before use.

1. With the Oxygen Software, enter the desired flow rate for the aqueous phase.

2. Once the flow rate of the aqueous phase is stable, enter the desired flow rate of the lipid phase.

3. Once the two flow rate are stable, you can start collecting the liposome phase. The stability of flow can also be checked by visualizing the entry of the herringbone mixer.

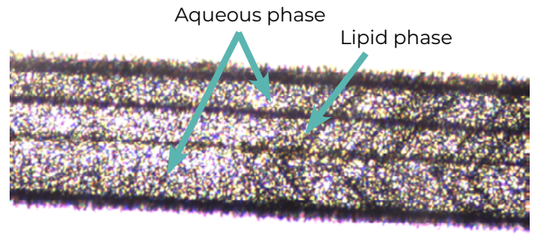


Figure 11: Stablization of aqueous and lipid phases inside the chip.

4. By changing the flow rate ratio of the phases and/or the total flow rate, the witdh of the flow of each phase is controlled. This can also be visualized at the inlet of the chip.

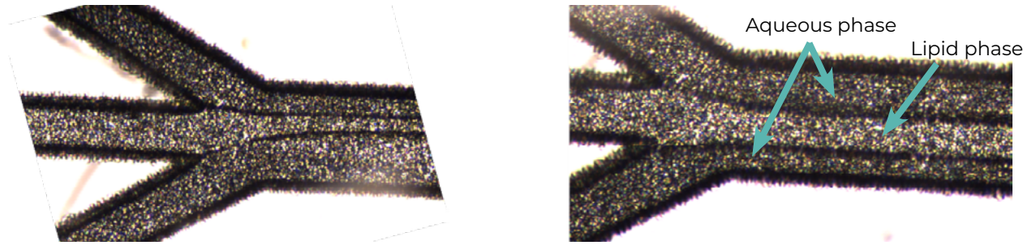


Figure 12: Impact of the flow rate ratio modification on the lipid and aqueous phases.

5. To stop de production, reduce the flow rate for each phase until it stops, then clean the chip by flushing the appropriate solvents (depending on the phase compositions). For this test, the chip was flushed with water, followed by ethanol, and water again. The chip can be reused.

Useful Tips:

- To encapsulate a drug or RNA, follow the same protocol while adding the drug in the appropriate phase:
  a) Add the drug to the aqueous phase if is is hydrophilic.
  b) Add the drug to the lipid phase if it is hydrophobic.
- Ensure that the flow of each phase is stable before collecting liposomes.
- Observe the entry and mixing channels to verify flow.
-Swtiching between several different lipid phase/samples is possible by adding microfluidic valves such as the 2-Switch or M-Switch to the setup.
-After collection, further purification depending on liposome composition and encapsulation, such as dialysis, may be performed. Store the liposomes appropriately before DLS or TEM analyses.

PROOF OF CONCEPT: LIPOSOME SIZE AND PDI IN FUNCTION OF THE FLOW RATE RATIO

The impact of the flow rate ratios (FRR) on liposome size was evaluated using the Liposome Production Pack.

$$FRR = \frac{Aqueous\ phase\ (DPBS)\ flow\ rate}{Organic\ phase\ (Ethanol)\ flow\ rate}$$

The FRR was varied (5:1, 4:1, 3:1) while keeping the total flow rate TFR constant (1 mL/min) at room temperature. For instance, at TFR = 1 mL/min and FRR = 3:1, the flow rate is 750 µL/min for the aqueous phase and 250 µL/min for the organic phase. Analysis using DLS showed liposome sizes from 40 and 150 nm depending on the conditions used. Generally, a decrease in liposome size was obtained with increasing FRR. A low PdI was measured for each size (figure below).

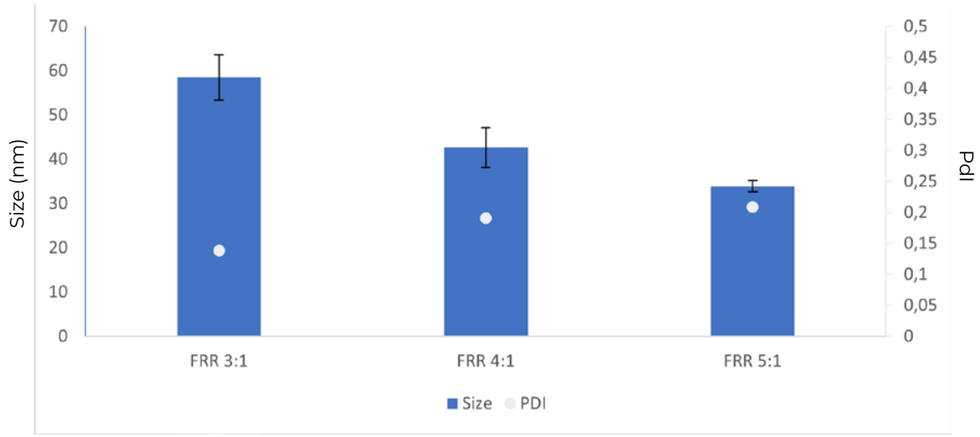


Figure 13: Liposome size as a function of FRR. Description of the size distribution by PdI.

To learn more about the effects of FRR, temperature, and drug encapsulation influence on liposome production, see the case study: Microfluidic Drug-Loaded Liposome production for Ovarian Cancer Treatment.

FAQ

1) How does one clean or unclog the chip?
If the entry or mixing channels are clogged, flush in the reverse direction (outlet to inlet) with the appropriate solvent (depending on the phase formulations). Increase the pressure of the Flow EZ to help evacuate the impurities/ aggregates from the clogged channel towards the inlet.
Always wear protective glasses when manipulating pressure.

2) How does one stop liquid leaks from occuring in the entry or exit port of the chip?
Check that the ferrules are appropriately connected to the tubing and fittings. Make sure that the fitting is totally plugged into the chip. If the leakage persists, try replacing the fitting and yellow-steel ring ferrule provided in the kit.

3) How are the Flow Units cleaded?
When cleaning and flushing Flow Units, consider the nature of the materials being pumped through them. Typically, one should select a cleaning solution that is safe for the Flow Unit (the inside surface). For Flow Units XLs, fluids must be compatible with PEEK & Borosilicate glass. Typically, flowing a 10% solution in water of RBS 25, Tergazyme or Alconox cleaners for 15 minutes at approx. 100 µL/min will restore performance.

4) What is the operational range for the Flow Unit?
The Flow Units are temprature compensated, and work over the range of 10°C to 50°C. It is highly recommended to verify the calibration the flow rate at extremes of the operating temperature range.

TECHNICAL SUPPORT

Any question? E-mail us at:

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