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Lab & Production Materials

NanoFabTx™ PEG Lipid Mix- for synthesis of PEGylated Liposomes

Protocol for Catalog No. 922420

Introduction

NanoFabTx™ nanoformulation kits and lipid mixes enable users to encapsulate a wide variety of therapeutic drug molecules for targeted or extended drug delivery without the need for lengthy trial-and-error optimization. ***NanoFabTx™*** kits provide an easy-to-use toolkit for encapsulating a variety of therapeutics in nanoparticles, microparticles, or liposomes. The resulting particles are biocompatible and biodegradable and can be further modified to target specific tissues or to ensure slow and sustained drug release. Drug encapsulated particles synthesized with the ***NanoFabTx™*** kits are suitable for biomedical research applications such as oncology, immuno-oncology, gene delivery, and vaccine delivery.

The kits and mixes minimize laboratory setup with optimized protocols and step-by-step instructions for synthesizing drug-encapsulated liposome-based formulations. Protocols for two different particle synthesis methods are included. One protocol uses the lipid film hydration/extrusion method to prepare liposomes using standard laboratory equipment. A protocol for microfluidics-based liposome synthesis using the Dolomite Microfluidics system is also included. The microfluidics protocol uses ***NanoFabTx™*** device kits, which provide the microfluidics chip, fittings and tubing required to get started with microfluidics-based synthesis and is compatible with the Dolomite Microfluidics system.

NanoFabTx™ **PEG Lipid Mix- for synthesis of PEGylated Liposomes** is designed for the synthesis of specifically sized PEGylated liposomes for hydrophilic drug encapsulation. The lipid mix contains rationally selected lipid in precise ratios that have been optimized to achieve a specific size range of liposomes. The synthesized PEGylated liposomes reduce protein absorption and prolongs circulation time. ***The NanoFabTx™*** **PEG Lipid Mix-for synthesis of PEGylated** liposomes includes a curated ready-to-use liposomes mix provides reagents and protocols for conventional lipid film hydration/extrusion and microfluidics methods to synthesize 80 nm to 120 nm liposomes.

Disclaimer

NanoFabTx™ **PEG Lipid Mix- for synthesis of PEGylated Liposomes** is for research use only; not suitable for human use. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Specifications

Storage	Store *NanoFabTx™ PEG Lipid Mix- for synthesis of PEGylated Liposomes* at -20°C. Protect from light.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials required for use with both methods, but not supplied

Catalog Number	Quantity
459836	Ethanol, 200 proof
276855	Dimethyl Sulfoxide (DMSO)
GE17-0851-01	Disposable PD 10 Desalting Columns
27024	Glass vials, clear glass (4 ml capacity)
V7130	Glass vial, clear glass (20 ml capacity)
09978	Ammonium sulfate, BioUltra, ≥99.0% (T)
SLFHX13	Syringe filters 0.45µm (for filtering non-aqueous solutions e.g. ethanol)
SLHAR33SS	Syringe filters 0.45µm (for filtering aqueous solutions e.g. buffer)
CLS430625	Corning® bottle-top vacuum filters, 0.45µm
PURX12050	Pur-A-Lyzer™ Maxi Dialysis Kit
D8537	Dulbecco's phosphate buffered saline (DPBS), no calcium, no magnesium (pH 7.4)

For Doxorubicin (Dox) remote loading of liposomes	
D1515	Doxorubicin hydrochloride or hydrophilic drug of choice

Materials required for use with the Dolomite Microfluidics system, but not supplied

Catalog Number	Description
911593	NanoFabTx™ microfluidic – nano device kit
	Pressurized pump system (protocol can be used with two or three pumps) (e.g. Dolomite Mitos P-Pump). Further information for compatible Dolomite Microfluidic pumps and microfluidic systems can be found at https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/.
	Dolomite flow sensors (protocol requires two flow sensors). Further information for compatible Dolomite Microfluidic flow sensors https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/.

Materials required for use with Extrusion, but not supplied

Catalog Number	Description
610000 ***or*** Z373400	Extruder Set with Holder Heating Block (Avanti Polar lipids) ***or*** LiposoFast Liposome Factory (Avestin)
Z373419	LiposoFast Liposome Factory-pore size 100 nm, polycarbonate membrane
Z373427	LiposoFast Liposome Factory-pore size 200 nm, polycarbonate membrane

Before you start: Important tips for optimal results

Filter solutions. For best results, filter the lipid solution through a 0.45 µm syringe filter (Cat. No. SLFHX13NL) before use. In addition, filter the buffer or water through a 0.45-µm syringe filter (Cat. No. SLHAR33SS) before use.

Lipid film hydration/extrusion method – The 50mg lipid mix (Cat No. 922420-50mg) will result in approximately 2 ml of liposomes solution upon hydration and extrusion whereas the 100mg lipid mix (Cat. No.922420-100mg) will make approximately 4 ml of liposomes solution.

Microfluidics method – Volume of collected nanoparticles. The volume of liposomes suspension can be controlled by adjusting the running/collection time. If only a small volume (1-2 ml suspension) of liposomes is required, run the microfluidics system for 2-3 mins. Similarly, for larger volume collect liposome suspension for ~5 minutes.

Microfluidics method – Reduce blockages with proper cleaning. Clean the microfluidics system after synthesis of each batch of liposomes. Improper cleaning can result in blockages in the micromixing microfluidics chip and tubing. A well-maintained microfluidics chip can be used multiple times through cleaning and proper storage.

Microfluidics method – Prime the tubing and chip. Prime the tubing and the micromixing microfluidics chip before starting liposome synthesis. Priming purges gases from the fluid pathways, conditions the chip surface, and serves as a check of chemical compatibility for all wetted parts of the system. In addition, priming reduces or prevents precipitation of lipids inside the system in the case of backflow, jetting, or chaotic mixing. Precipitation of reagents can irreversibly block the microfluidics chip.

Microfluidics method – Using the Dolomite Microfluidics System. The flow rates listed in the protocol are optimized using the Dolomite Microfluidics pressurized pump system with flow sensors attached. If a syringe pump is used instead of pressurized pumps, the size of the liposomes and percentage of drug loading could deviate from the protocol.

Procedure

Two procedures to synthesize PEGylated liposomes are provided below: **Procedure 1** –**Lipid film hydration and extrusion method** and **Procedure 2** –**microfluidics-based method**. **Procedure 1** is a widely used method to prepare liposomes because of its scalability, simplicity, and lack of requirements for expensive laboratory instruments. **Procedure 2** is a microfluidics or continuous flow-based technique that results in narrow size distribution, enhanced control over each stage of particle fabrication, greater particle yields, ease of scalability, and excellent reproducibility. This microfluidics-based protocol uses the rapid micromixing method to synthesize liposomes.

This procedure for hydrophilic drug encapsulation was developed and optimized to achieve a desired size range using doxorubicin (Dox) as a model. The *NanoFabTx™-PEG Lipid Mix, for synthesis of PEGylated liposomes* protocol can be modified for other therapeutic agents of interest. The procedure below is suggested as a guide for your own optimization.

Prepare reagents

1. Prepare Ammonium sulfate (AS) buffer (240 mM, pH 5.4)

- Dissolve 31.71 g of ammonium sulfate salt in 1 liter of deionized water.
- The pH of the solution is natively 5.4, additional pH adjustment is not required
- Filter the AS buffer through a 0.45-µm filter before use (Cat. No. CLS430625).

Procedure 1 – Prepare liposomes by hydration/extrusion method

Lipid extrusion is a technique in which a lipid suspension is forced through a polycarbonate filter with a defined pore size. Liposome size is determined by the pore size of the filter (ie 100nm pore size filter results in approximately 100nm diameter liposomes). The extrusion process can be carried out with the suggested list of extruders in the materials table. The following protocol was optimized for liposome preparation using the Extruder Set with Holder Heating Block (Cat. No. 610000). See Figure 1 for more details.

1. Prepare lipid mix solution

- Remove the crimp seal/septum from the lipid mix vial and add AS buffer to a final concentration of 25mg/ml (2ml for 50mg, 4mL for 100mg)
- Vortex the solution for 5 mins to completely dissolve the lipid mix into the buffer.
  Note: Mild sonication can also be used to completely dissolve the lipid mix.

2. Prepare multi-layer vesicles (MLVs)

- Place the vial containing lipid mix solution in a water bath or incubator set at 65°C.
- Gently shake for 1 hour at 65°C.
- Complete five freeze-thaw cycles: 3 minutes on ice and 3 minutes in a 65°C water bath to reduce MLV size.

3. Assemble the extruder

- Assemble the extruder per manufacturer recommendations.

  Note: The extrusion process can be carried out with the suggested list of extruders in the materials table. The following protocol was optimized for liposome preparation using the Extruder Set with Holder Heating Block (Cat. No. 610000)

- Assemble extruder with a 200 nm polycarbonate membrane (Cat No. Z373427)
- Keep the assembled extruder on a hot plate to maintain temperature at 65 °C.

  Note: Use caution, do not touch hotplate during the extrusion process.

4. Extruder liposomes

- Load 1 ml of MLV solution in an extruder syringe (Syringe 1). Attach the syringe to one side of the extruder assembly.
- Attach an empty syringe (Syringe 2) on the other side of the extruder.

Figure 1. *The lipid extrusion process. The MLV solution in Syringe 1 is transferred to Syringe 2 by gently pushing on the plunger of Syringe 1. The solution is then transferred back to Syringe 1 by gently pushing on the plunger of Syringe 2. This process is repeated for a total of 11 cycles.*

- Gently push the plunger of Syringe 1 to completely transfer the MLV solution from Syringe 1 to Syringe 2 (Figure 1).
- Gently push the plunger of Syringe 2 to completely transfer the solution back to the original syringe (Syringe 1). This completes one cycle.
  Note: One cycle is defined as transferring the solution from one syringe to the next, and back to the original syringe.
- Repeat the above steps 10 more times (for 11 total cycles). The solution should be completely transferred to Syringe 2 after the final pass-through.
- Remove the extruder from the block.
- Replace the 200nm polycarbonate membrane (Cat No. Z373427) with the 100nm polycarbonate membrane (Cat No. Z373419). The MLV solution should remain in Syringe 2.
- Gently push the plunger of Syringe 2 to completely transfer the MLV solution to Syringe 1.
- Gently push the plunger of Syringe 1 to completely transfer the MLV solution to Syringe 2. This completes one cycle.
- Repeat the above steps 10 more times (for 11 total cycles). The solution should be completely transferred to Syringe 1 after the final pass-through.
- Remove the extruder from the block.
- Remove the syringes from the extruder.
- Inject the lipid solution into a clean vial (approximately 1 mL) and store at 4°C until further use.
- For immediate remote drug loading, please proceed to the “Remote loading of Doxorubicin into liposomes” section.
- For empty liposomes, centrifuge at 30,000-60,000 x g for 30 minutes to 1 hour to collect liposome pellets. If desired, liposomes can be lyophilized for long-term storage.

5. Clean the extruder

- Wipe the extruder apparatus with isopropyl alcohol and thoroughly dry it before storing.
- Rinse the syringes with isopropyl alcohol followed by DI water and thoroughly dry before storing.

Note: Membranes are intended to be used for a single liposome preparation and should not be reused

6. Measure liposome size

- Measure the size of the liposomes with a dynamic light scattering instrument and transmission electron microscopy (TEM).

Procedure 2 – Microfluidics-based method to synthesize PEGylated liposomes

A. Set up the microfluidics system

This microfluidics protocol is designed for use with the corresponding *NanoFabTx™ Microfluidic - Nano* Device kit (Cat. No. 911593) which contains all required device components for synthesizing liposomes and a detailed protocol for use with a Dolomite Microfluidics system. Please follow the protocol with *NanoFabTx™ Microfluidic - Nano* Device kit (Cat. No. 911593) to set up the microfluidics system. **Figure 2** shows a schematic of the microfluidics setup and tubing connections for a Dolomite Microfluidics system.

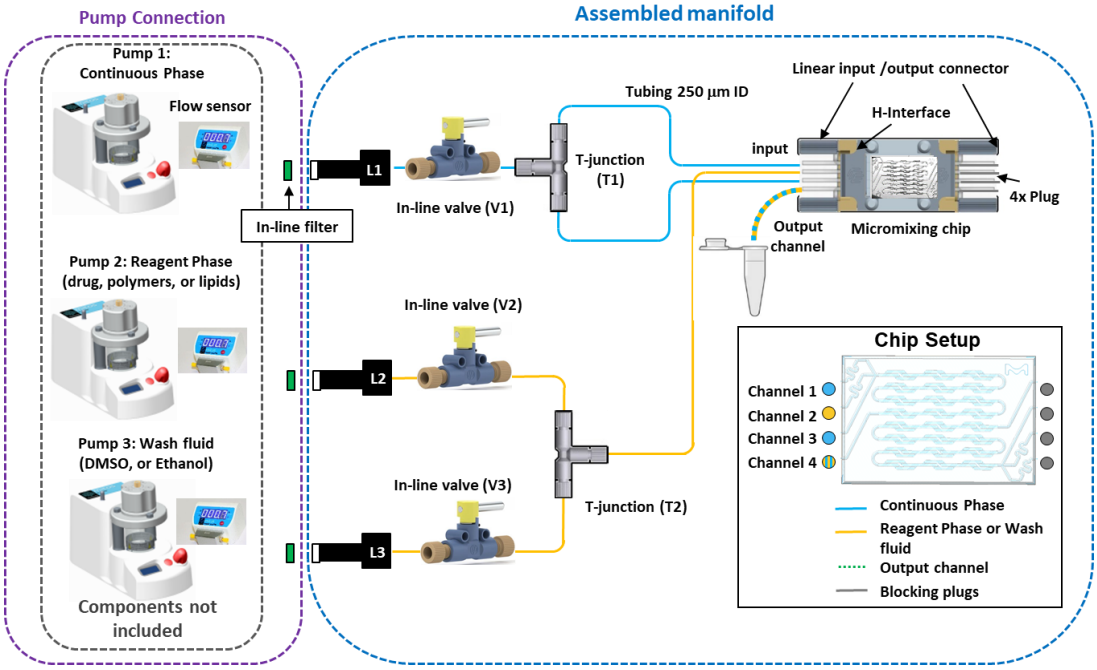

Figure 2: *Schematic of the microfluidics setup for the Dolomite Microfluidics system. The manifold is supplied preassembled. The microfluidics chip is packaged separately. In-line filters are supplied for connection to microfluidic pumps. More information can be found in the corresponding NanoFabTx™ Microfluidic - nano Device kit (Cat. No. 911593).*

B. Prepare liposomes by microfluidics

1. Filter AS buffer

- Filter 10 ml of AS buffer using syringe filter (Cat. No. SLHAR33SS) and add to a clean glass vial (Cat. No. V7130)

2. Prepare lipid mix solution

- Remove the crimp seal/septum from the lipid mix vial and add ethanol (200 proof) for a final concentration of 25mg/ml (2 ml for 50mg, 4ml for 100mg).
- Vortex the solution to completely dissolve the lipid mix
- Filter the lipid solution using a syringe filter (Cat. No. SLFHX13NL) into a 4 ml glass vial (Cat. No. 27024)

3. Assemble microfluidics system

- Assemble the microfluidics system as described in protocol with *NanoFabTx™ Microfluidic - Nano* Device kit (Cat. No. 911593)

  Note: The synthesis of liposomes using the Dolomite Microfluidics system can be carried out in either a three-pumps (as shown in **Figure 2**) or two-pumps configuration (pump 1 and 2 only). In the three-pumps configuration, a vial of priming solution is kept in pump 3 throughout the process. Pump 3 can be used for the washing step without the need for swapping the vials in pump 2 when using a two-pumps configuration. For washing, simply close valve V1 and V2 and open valve V3 and start the flow of DMSO using the pump software.

4. Insert priming solvent DMSO into microfluidics system

- As shown in the setup illustrated in **Figure 2,** place a vial (Cat. No. V7130) of DMSO inside pump 1. Pump 1 is connected to channels 1 and 3 of the micromixing microfluidics chip.
- Place another vial (Cat. No. V7130) of DMSO inside pump 2 or pump 3. Pump 2 and pump 3 are connected to the channel 2 of the micromixing microfluidics chip via T-junction (T2).
- Keep valves V1, V2, and V3 closed. They connect to pump 1, pump 2, and pump 3 respectively.

5. Prime the system

- Place a waste collection vial at the output channel to collect waste generated during setup and priming.
- Open valve V2 to flush the assembled manifold with the DMSO (no lipid solution) by setting a flow rate of 100 µl/min for pump 2 using the Flow Control Center software for the Dolomite microfluidics system. Keep valve V3 closed if using the two-pumps configuration.
- Close valve V2 and open valve V1 to flush the assembled manifold with DMSO by setting a flow rate of 100 µl/min for pump 1. For further details on priming the system refer to the device kit protocol (Cat. No. 911593)

6. Prepare liposomes

- Place the vial (Cat. No. V7130) containing 10 ml of filtered AS buffer inside pump 1 and the vial (Cat No 27024) containing 2 ml of lipid mix in ethanol inside pump 2.
- Check that valves V1, V2, and V3 are closed. Always keep valve V3 closed when using the two-pump configuration.
- Select the flow rates on pump 1 and 2 from **Table 1** based on desired liposome size. An initial flow rate of 50ul/min for reagent phase and 500ul/ml for buffer phase is suggested. Increasing flow rate ratio (buffer phase/reagent phase) decreases liposome size. A flow rate ratio range between 2 to 20 is suggested.

Table 1: Flow rate settings for preparing liposomes

Desired Liposomes size (nm)	Flow rate ratio	Flow rate – Pump 2 Reagent phase (lipid mix) (ul/min)	Flow rate – Pump 1 Buffer phase (AS buffer) (ul/min)
90	**5**	**50**	250
98	**10**	**50**	500
98	**20**	**50**	1000

- Open valve 1 and set the flow rate of pump 1 (AS buffer) based on **Table 1** values using the Flow Control Center software of the Dolomite microfluidics system.

- Set the flow rate for pump 2 (lipid mix solution) based on **Table 1** values using the Flow Control Center software of the Dolomite microfluidics system.
- The flow rates of both solutions will stabilize within a few seconds. Optional: fluid flow of the two solutions can be visualized by a high-speed microscope.
- After the flow rates of the two solutions have stabilized, replace the waste collection vial with a sample collection vial at the output channel and collect the liposome suspension.
- When you have collected the desired volume of the liposome suspension, transfer the output channel tubing to the waste collection vial, close valves V1 and V2, stop fluid flow using the Flow Control Center software and remove the solution vials from pump 1 and pump 2.

7. Remove excess ethanol from liposome suspension

- Transfer 1-2 ml of liposomes suspension into a dialysis cassette (Cat. No. PURX12050) and dialyze the samples against 1L of AS buffer for at least 4 hrs. Collect the purified samples and store at 4°C until further use.

***Note*:** Buffer selection for dialysis depends on the pKa of the encapsulated drug and AS buffer may not be suitable for all drugs.

- Measure the size of the liposomes with a dynamic light scattering instrument and transmission electron microscopy (TEM).
- For immediate remote drug loading, please proceed to the “Remote loading of Doxorubicin into liposomes” section.
- For empty liposomes, centrifuge at 30,000-60,000 x g for 30 minutes to 1 hour to collect liposome pellets. If desired, liposomes can be lyophilized for long-term storage.

8. Clean the microfluidics system

Note: Clean the microfluidics system after each use using the method below. Improper cleaning can result in chip and tubing blockage.

- Follow this cleaning procedure after each run to remove any remaining lipid precipitates or deposited buffer.
- Use DMSO to clean the tubing and micromixing microfluidics chip. DMSO is the preferred cleaning solvent, because both the lipids and buffer have high solubility in DMSO.
- Filter 10 ml DMSO through a 0.45 µm syringe filter into each of two vials (Cat. No. V7130).
- Close valves V1, V2, and V3 and place a waste collection vial at the output channel tubing.
- Place the vials of filtered DMSO in pumps 1 and 2.
- Open valve V1 and set the flow rate of pump 1 to 100 µl/min.
- Set the flow rate of pump 2 to 100 µl/min and immediately open valve V2.

Note: If using a three-pump configuration, washing is not required for pump 3.

- Gradually increase the flow rate on both pumps to 300 µl/min. Run the system for 3 minutes to completely remove any lipids, or buffer precipitated inside tubing or micromixing microfluidics chip.
- When the cleaning process is complete, close valves V1 and V2 and use the software or pump interface to immediately stop the flow of the liquids through pumps 1 and 2.
- Remove the DMSO vials.
- Disconnect the linear input/output connectors and remove the micromixing microfluidics chip from the H-interface.
- Store the micromixing microfluidics chip in its box or another clean, dust-free environment.

Remote loading of Doxorubicin into liposomes

The transmembrane pH gradient method is widely used for loading hydrophilic drugs like doxorubicin into liposomes. This is a generalized method (for guidance only) that has been optimized for the model drug Doxorubicin (Dox) (D1515). Modification of the below methods is required for encapsulation of other hydrophilic drugs.

1. Create a pH gradient through external buffer exchange
    - Prepare PD-10 desalting columns (cat. No. GE17-0851-01) as per manufacturer recommendations using phosphate buffered saline (PBS) (pH 7.4) as the equilibration buffer.
    - After the column is equilibrated with PBS, add 2 ml of liposomes suspension to the column.
    - Collect the eluate by centrifuging the column at 1000 x g for 2 minutes.
2. Prepare Drug (Dox) Solution
    - Dissolve 10 mg of Dox in 1mL of PBS for a final concentration of 10 mg/ml.
3. Remote Dox loading into liposomes
    - Add 2 ml of the buffer-exchanged liposomes to a new glass vial (Cat No 27024).
    - Add 250 µl of 10 mg/ml Dox solution to the liposome solution.

    Note: For loading Dox inside liposomes, a Dox to lipid weight ratio of 1:20 is suggested. For example, for 2ml of 25mg/ml liposome solution, add 250 µl of 10 mg/ml doxorubicin solution. The suggested final Dox concentration should be 1.25 mg/ml.

    - Gently stir the solution on a temperature-controlled stir plate using a magnetic stir bar for 1 hour at 60°C. Set the stirring speed to approximately 300 rpm.
4. Remove unencapsulated drug
    - Prepare PD-10 desalting columns (cat. No. GE17-0851-01) as per manufacturer recommendations using PBS (pH 7.4) as equilibration buffer.
    - After the column is equilibrated with PBS, add 2 ml of Dox-loaded liposomes to the column.
    - Collect the eluate by centrifuging the column at 1000 x g for 2 minutes.
    - Store Dox-loaded liposomes at 4°C in PBS for further use.

Quantify drug encapsulation in liposomes

The method for quantifying drug encapsulation in liposomes varies depending on the drug. This is a generalized HPLC method (for guidance only) that has been optimized for the model drug doxorubicin (Dox). Modification of the below methods is required based on the encapsulated drug and method chosen for quantification.

1. Extract encapsulated drug from prepared liposomes
    - Mix liposome samples with 50% isopropyl alcohol in order to disrupt the liposome membrane and release entrapped drug content.

2. HPLC quantification

- After liposome disruption, the free drug can be quantifying using HPLC.
- Take 200 ul of the disrupted liposome solution and add an equal volume of acetonitrile.

***Note*:** The recommended mobile phase for Dox is 50:50 acetonitrile/water. The optimal mobile phase may vary for your drug of interest.

- Quantify drug content using HPLC.

3. Quantification analysis

- *Encapsulation efficiency* (EE%) is the percentage of drug that is encapsulated into the liposomes.

  Encapsulation efficiency is calculated by:

$$EE\ (\%) = \frac{Encapsulated\ drug}{Total\ drug\ added} x\ 100$$

- *Loading capacity* (LC%) is the amount of drug-loaded per unit weight of the lipid and indicates the percentage of the mass of lipids that contains the encapsulated drug.

  Loading capacity is calculated by:

$$LC(\%) = \frac{Total\ amount\ of\ encapsulated\ drug}{Total\ weight\ of\ lipids}\ x\ 100$$

Troubleshooting

Detailed troubleshooting on the microfluidics setup is provided in the troubleshooting guide included in the *NanoFabTx™ Microfluidic - Nano, device kit* (Cat. No. 911593). Due to the numerous connections between microfluidics components, and the narrow flow paths for the fluids, you may encounter leaks or blockages. This section presents information on and potential solutions for commonly encountered problems.

1. Liposomes are not in the desired size range

Possible cause – This protocol is optimized for synthesis of liposomes in the 80 nm to 120 nm using the model drug, doxorubicin. If you encapsulate a different drug in liposomes, the size of your liposomes may vary from the size range reported here.

Solution – If using the film/extrusion method, membrane filters of different sizes (200 nm, 100 nm, etc) are commonly used to control final liposome size. Be sure to select the correct membrane filter for your targeted liposome size.

Possible cause – Procedure 2 is optimized for synthesis of liposomes in the size ranges of 80 nm to 100 nm using the Dolomite microfluidic system. The flow rates listed in the protocol are optimized using pressurized pumps with flow sensors attached. If you use different flow rates, or alternative syringe pumps, the size of your liposomes may vary from the size range reported here.

Solution – If using the microfluidics method, liposome size can be tuned by changing the flow rate ratio (reagent phase: buffer phase). Increasing the flow rate ratio decreases liposome size.

2. Polydisperse liposomes

PDI is the standard deviation of the particle diameter distribution divided by the mean particle diameter. PDI is used to estimate the average uniformity of a particle solution; higher PDI values correspond to a greater size distribution in the nanoparticle sample. A sample is considered monodisperse when the PDI value is less than 0.1

Possible cause – Polydisperse samples can result from improper extrusion process.

Solution – For extrusion, ensure the correct membrane filters are used and the correct number of passes through the extruder are completed to minimize polydispersity.

Possible cause – In the microfluidics method, polydisperse samples can occur if the flow in the tubing or micromixing microfluidics chip is uneven or blocked.

Solution – The next sections provide tips to minimize uneven flow or remove blockages.

3. Uneven flow in the microfluidics-based method

Possible cause – Uneven flow can be caused by bubbles of air in the system.

Solution – Fluid flowing through the system will clear bubbles within 1–2 min. You can usually see the bubbles passing through the micromixing microfluidics chip. If this approach does not remove the bubbles, sonicate the solutions for 30 min and vent the pressure chamber.

Possible cause – If the flow becomes unstable when the microfluidics system has been in operation for a while, one of the solution supplies may have run dry or the pick-up tubing might not reach to the bottom of a vial.

Solution – Check that the vials contain enough reagent and that the 250-µm pick-up tubing is long enough to collect from the bottom of each vial.

Possible cause – If none of the above solutions leads to even flow, the software may need to be rebooted.

Solution – Stop all flow, close and reopen the Flow Control Centre software, and restart flow. If this method does not solve the problem, the system may have a blockage. Check for blockages as detailed in the next section.

Possible cause – If the system has no blockages, the flow sensor may not function correctly.

Solution – Replace the flow sensor.

4. Leak in system

Possible cause – Changes/fluctuations in system pressure or flow rate can arise from a leak in the system.

Solution – Before troubleshooting a possible blockage, make sure that all connectors are properly fitted and that the system has no apparent leaks.

5. Blockage of tubing or micromixing microfluidics chip

Possible cause – During the synthesis of liposomes using the microfluidics setup, the introduction of dust fibers, deposition of precipitated buffer/lipids, or drying of buffer/lipids inside the micromixing microfluidics chip or tubing, or improper cleaning procedures can cause blockage in the micromixing microfluidics chip or tubing.
Several indications suggest that a partial or complete blockage has occurred:

a. Consistent flow rate is maintained when a pump is in flow control mode, but the pressure increases.
b. Consistent pressure is maintained when a pump is in pressure control mode, but the flow rate decreases.
c. The instrument software has set changes to the flow rate, but apparent flow rate does not change.
d. The flow is significantly slower than expected.
e. The flow rate fluctuates unexpectedly and affects droplet stability.

Possible cause – If a partial or transitory blockage is present, the pressure may increase gradually, then suddenly drop as the blockage moves along the flow path, and then increase again when the obstruction becomes lodged.

Solution – Blockages can occur anywhere in the flow path of the system; identifying the location of a blockage is a process of elimination.

Start with the micromixing microfluidics chip, because sometimes blockages (dust or hair) are visible under a microscope. If you find a blockage on the chip, monitor it while you vary the pump pressure to try to dislodge it. If a blockage on the chip cannot be cleared, the chip will need to be replaced.

If you see no physical blockage in the micromixing microfluidics chip, disconnect the chip interface and check whether liquid flows from the tubing. If liquid now flows from the disconnected tubing the blockage is likely either in the chip or the connector was improperly seated against the chip. If the system has a T-connector that splits the flow of a solution into two inputs, check that the flow rates through each input are identical. If the flow is asymmetric, a blockage could be somewhere between the T-connector and the chip. First replace the tubing and see if this fixes the problem; if not, replace the T-connector.

If it is not already apparent which line is blocked, vary the flow rate of the solutions one at a time while observing the ends of the tubing. This step will help to identify which line is blocked.

Work your way back through the system, from the chip to the pump, one component at a time, and check for stable flow at each stage. When you find the section that contains the blockage, simply replace it.

The blockage may have occurred because of particulate contamination in your solution(s). Refilter solutions through a 0.45µm syringe filter before use.