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Lab & Production Materials

Protocol

NanoFabTx™ DBCO Lipid Mix, for synthesis of DBCO functionalized liposomes

Protocol for Cat No #934208

Introduction

NanoFabTx™- DBCO Lipid Mix, for synthesis of DBCO functionalized liposomes is designed for the synthesis of specifically sized liposomes surface-functionalized with DBCO groups for targeting and immunotherapy applications, and small molecule encapsulation. The lipid mix contains rationally selected lipids in precise ratios that have been optimized to achieve a specific size range of liposomes.

The synthesized DBCO-functionalized liposomes enable 1) selectively targeting cells which overexpressed azide-tagged receptors; 2) the conjugation of azide-tagged antibodies or proteins to the liposome surface, thus introducing immune reactions or multi-targeting. The ***NanoFabTx*™- DBCO Lipid Mix, for synthesis of DBCO functionalized liposomes** includes a curated ready-to-use lipid mix and step-by-step protocols for conventional lipid film hydration/extrusion and microfluidics methods to synthesize 80 nm to 120 nm liposomes.

Disclaimer

NanoFabTx™- DBCO Lipid Mix, for synthesis of DBCO functionalized 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™-Biotin Lipid Mix, for synthesis of biotin functionalized 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, but not supplied

Catalog Number	Name
459836	Ethanol, 200 proof
276855	Dimethyl Sulfoxide (DMSO)
09978	Ammonium sulfate, BioUltra, ≥99.0% (T)

Prepare reagents:

1. Prepare ammonium sulfate (AS) buffer (240 mM, pH 5.4)
   - Dissolve 31.71g of ammonium sulfate salt in 1L of deionized water
   - The pH of the solution is 5.4, additional pH adjustment is not required
   - Filter the AS buffer through a 0.45µm filter before use (Cat.No. **CLS430625**)

Procedure

Procedure 1- Preparation by microfluidics

The following protocol was designed for use with the NanoFabTx™ microfluidic-nano device kit (Cat.No. **911593**). Please refer to the protocol provided with the NanoFabTx™ microfluidic-nano device kit (Cat.No. **911593**) for microfluidics assembly.

1. Prepare filtered AS buffer using a syringe filter (Cat. No. **SLHAR33SS**) and transfer to clean glass vial (Cat.No. **V7130**) or syringe.
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.
   - Gently vortex the solution until completely dissolved.
   - Filter the lipid solution using a syringe filter (Cat. No. **SLFHX13NL**) into a 4ml glass vial (Cat.No. **27024**) or syringe.
3. Prime the system
   - Keeping valves V1,V2, and V3 closed, place a syringe or vial of DMSO in pump 1. Connection pump 1 to channels 1 and 3 of the microfluidics chip.
   - Place another syringe or vial of DMSO in pump 2. Connect pump 2 to channel 2 of the microfluidics chip.
   - Place a waste collection vial at the output channel to collect waste generated during setup and priming.
   - Open valve V2 and set the flow rate of pump 2 to 100µl/min to prime the chip.
   - Stop pump 2, close valve V2, open valve V1, and set the flow rate of pump 1 to 100µl/min to prime the chip.
   - Stop pump 1, close valve V1, and remove DMSO syringes or vials from pumps 1 and 2. More information on priming can be found in the device kit protocol.
4. Prepare liposomes by microfluidics
   - Place a vial or syringe containing 10ml of filtered AS buffer inside pump 1 and the vial or syringe containing the lipid mix solution inside pump 2.
   - Check that valves V1, V2, and V3 are closed. Always keep valve V3 closed for a two-pump configuration (refer to device kit protocol for more information).
   - Select the flow rates for pump 1 and pump 2 from **Table 1**.
     Note: An initial flow rate of 50µl/min for the lipid solution and 500µl/min for the buffer is suggested. Increasing the flow rate ratio (lipid solution: buffer) decreases liposome size. A flow rate ratio between 1:2 and 1:20 is suggested.
   - Open valve 1 and set the flow rate of pump 1 (buffer) to that recommended in Table 1.
   - Set the flow rate of pump 2 (lipid solution) to that recommended in Table 1, and open valve 2. The flow rates of both solutions will stabilize within a few seconds.
   - 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, transfer the output channel to the waste collection vial, close valves V1 and V2, stop pumps 1 and 2, and remove the vials or syringes from pumps 1 and 2.
5. Remove excess ethanol from liposome suspension.
   - Transfer 1-2ml of liposome suspension into a dialysis cassette (Cat.No. PURX12050) and dialyze the samples against 1L of AS buffer for at least 4 hours. Collect the purified samples and store at 4°C until further use.
6. Clean microfluidics system
   - After each run, use syringe filtered DMSO to clean the tubing and microfluidics chip.
   - Place 10ml of filtered DMSO in pumps 1 and 2 and place a waste collection at the output channel.
   - Open valve V1 and set the flow rate of pump 1 to 100µl//min.
   - Set the flow rate of pump 2 to 100µl//min and open valve V2.
   - Gradually increase the flow rate of both pumps to 300µl//min and run the system for 3 minutes.
   - When the cleaning process is complete, close valves V1 and V2, stop the pumps, and remove the DMSO.
   - Disconnect the input connectors from the pumps and remove the microfluidics chip from the manifold. Return the microfluidics chip and manifold to its box and place in a clean, dust-free environment.

Procedure 2- Preparation by hydration/extrusion

The following protocol was optimized for liposome preparation using the Extruder Set with Holder Heating Block (Cat.No. **610000**).

1. Prepare lipid mix solution
   - Remove the crimp seal/septum from the lipid mix vial and add AS buffer for a final concentration of 25mg/ml.
   - Gently vortex the solution until completely dissolved.
     Note: Mild sonication or heating (65°C) can aid the dissolving process.
2. Prepare multi-layer vesicles (MLVs)
   - Place the vial containing the lipid mix solution in a water bath or incubator set at 65°C and gently share for 1 hour.
   - Complete five freeze-thaw cycles – 3 minutes on ice and 3 minutes at 65°C to reduce MLV size.
3. Assemble the extruder per manufacturer recommendations using a 200 nm polycarbonate membrane (Cat. No. **Z373427**). Keep assembled extruder on a hot plate to maintain 65°C.
4. Prepare liposomes by extrusion.
   - Load 1ml of MLV solution in an extruder syringe (Syringe 1) and attach the syringe to one side of the extruder assembly. Attach an empty syringe (Syringe 2) to the other side of the extruder.
   - Gently push the plunger of Syringe 1 to completely transfer the MLV solution from Syringe 1 to Syringe 2. Gently push the plunger of Syringe 2 to completely transfer the solution back to Syringe 1. This completes one cycle. Repeat 10 more times for a total of 11 cycles. At the final pass-through, the solution should be completely transferred to Syringe 2.
   - Remove the extruder from the block and replace the 200nm polycarbonate membrane with the 100nm polycarbonate membrane (Cat.No. **Z373419**) and attached syringe 1 and 2 to the extruder assembly. The MLV solution should remain in Syringe 2.
   - Once reassembled, gently push the plunger of Syringe 2 to completely transfer the MLV solution to Syringe 1. Gently push Syringe 1 to completely transfer the MLV solution to Syringe 2. Repeat 10 more times for a total of 11 cycles. At final pass-through, the solution should be completely transferred to Syringe 1.
   - Transfer the liposome solution to a clean vial and store at 4°C until further use.
5. Clean extruder
   - Wipe the extruder apparatus with isopropyl alcohol and thoroughly dry before storing. Rinse the syringes with isopropyl alcohol followed by DI water and thoroughly dry before storying. Membranes are intended for single use only.

Table 1. Suggested flow rates for microfluidics preparation of liposomes

Flow rate ratio	Lipid solution flow rate (µl/min) (pump 2)	Buffer flow rate (µl/min) (pump 1)
1:5	50	250
1:10	30	300
1:20	50	1000

Liposome usage and storage:

Liposomes can be used immediately following preparation. For long-term storage of empty liposomes, centrifuge at 30,000-60,000xg for 30 minutes to 1 hour to concentrate liposome pellet and lyophilize.

Physiochemical characterization of liposomes:

Liposomes and lipid nanoparticles are usually characterized in terms of size, morphology, zeta potential, drug content and cytotoxicity. A wide range of techniques are available for the physiochemical characterization of nanoparticles and some of the most used techniques are listed in table 2.

Table 2. Principle techniques for physiochemical characterization of nanoparticle

Parameter	Technique
Size and morphology	Dynamic light scattering Transmission electron microscopy Scanning (electron, force) microscope
Zeta potential	Dynamic light scattering
Drug content	High-performance liquid chromatography Ultraviolet-visible spectroscopy
Cell uptake and cytotoxicity	LDL uptake assay MTT cytotoxicity assay LDH cytotoxicity assay