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Protocol

NanoFabTx™ gene delivery, azide lipid mix

Protocol for Cat No # 937231

Introduction

NanoFabTx™- gene delivery, azide lipid mix is designed for the synthesis of specifically sized lipid nanoparticles (LNPs) surface-functionalized with azide groups for targeting and immunotherapy applications, and nucleic acid encapsulation. The lipid mix contains rationally selected lipids in precise ratios that have been optimized to achieve a specific size range of LNPs. The synthesized azide-functionalized LNPs enable the conjugation of alkyne-tagged (*e.g.*, dibenzocyclooctynes (DBCO) and difluorocyclooctynes (DIFO)) antibodies or proteins to the LNPs surface, thus introducing immune reactions or multi-targeting. The **NanoFabTx™- gene delivery, azide lipid mix** includes a curated ready-to-use lipid mix and step-by-step protocols for microfluidics methods to synthesize 80 nm to 200 nm liposomes. The table below can be used to help you determine the lipid concentration and azide group concentration.

Table 1. Total lipids and maleimide groups (moles)

	TOTAL LIPIDS (µMOLE)	AZIDE GROUP (µMOLE)	CATIONIC LIPIDS (µMOLE)
CAT NO. 937231-20mg	32.47	0.33	4.22

Disclaimer

NanoFabTx™- gene delivery, azide lipid mix is for research use only; not suitable for human use. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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 1ml of nucleic acid solution and transfer to syringe.
   - Take mRNA sample (1mg/mL) out from -80 ℃ refrigerator and thaw it on ice.
   - Take 28 ul of mRNA stock into a centrifugation tube.
   - Add 1022 ul of sodium citrate buffer (100mM, pH=4.0).
   - Store the mRNA solution on ice and use within 24-48 hrs.

   Note: CleanCap® FLuc mRNA (Tri-Link Biotechnologies, Cat# L-7202) was used as a model with N/P ratio of 10:1. N/P ratio may need to be optimized depending on the length of RNA or DNA.

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 5mg/mL.
   - Gently vortex the solution until completely dissolved.
   - Filter the lipid solution using a syringe filter (Cat. No. SLFHX13NL) into a syringe.

3. Prime the microfluidics system
   - Keeping valves V1,V2, and V3 of the microfluidic device closed, place a syringe of DMSO in pump 1.
   - Connect pump 1 to channels 1 and 3 of the microfluidics chip.
   - Place another syringe 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 from pumps 1 and 2.

   Note: More information on priming can be found in the device kit protocol.

4. Prepare LNPs by microfluidics
   - Place a syringe containing 1ml nucleic acid solution inside pump 1 and the 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 2**.

Note: An initial flow rate of 50µl/min for the lipid solution and 250µl/min for the nucleic acid is suggested. Increasing the flow rate ratio (lipid solution: nucleic acid) decreases LNPs size. A flow rate ratio between 1:3 and 1:10 is suggested.

- Open valve 1 and set the flow rate of pump 1 (nucleic acid solution) to that recommended in Table 2.
- Set the flow rate of pump 2 (lipid solution) to that recommended in Table 2, 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 syringes from pumps 1 and 2.

5. Remove excess ethanol from LNPs suspension.
   - Transfer all LNPs suspension into a dialysis cassette (Cat.No. PURX12050) and dialyze the samples against 1L of icy PBS 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 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.

Table 2. Suggested flow rates for microfluidics preparation of LNPs

Desired LNPs size (nm)	Flow rate ratio	Nucleic acid flow rate (µl/min) (pump 1)	Lipid solution flow rate (µl/min) (pump 2)
150	1:5	250	50

Lipid nanoparticles usage and storage:

Lipid nanoparticles can be used immediately following preparation. For long-term storage of empty LNP, centrifuge at 30,000-60,000xg for 30 minutes to 1 hour to concentrate LNP pellet and lyophilize. The obtained LNP should be stored at 4 ℃.

Application Note

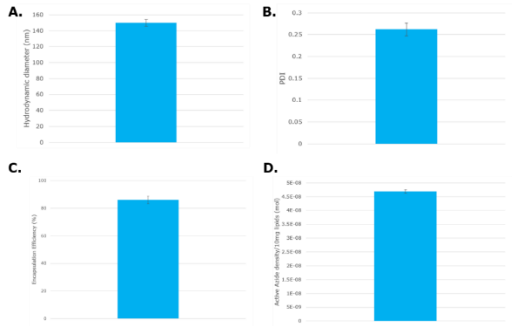

Figure 1. A) Hydrodynamic diameter of lipid nanoparticles, B) Polydispersity (PDI), C) mRNA encapsulation efficiency, D) Azide density.

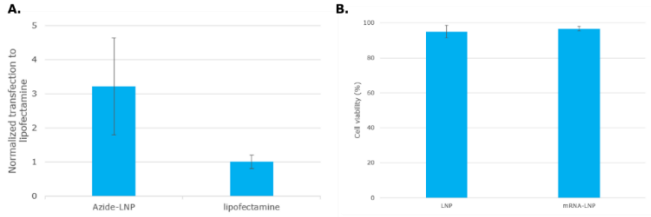

Figure 2. A) mRNA transfection efficiency characterized by cell expression of luciferase, B) cell viability of azide -functionalized LNPs and mRNA-loaded azide functionalized LNPs.