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Lab & Production Materials

Protocol

NanoFabTx™ NanoFlash PEG lipid mix
For CIJ synthesis of PEGylated liposomes
Protocol for Cat No # 933589

Introduction

This kit is designed for the synthesis of specifically sized PEGylated liposomes for hydrophilic drug encapsulation. The NanoFlash PEG lipidmMix 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 PEG Lipid Mix-for synthesis of PEGylated liposomes includes a curated ready-to-use liposomes mix and provides reagents and protocols for flash nanoprecipitation method using a Confined Impinging Jet (CIJ) to synthesize nanoparticles ranging from 50 nm to 150 nm in size. This allows users to select the optimal conditions to achieve the desired drug loading and nanoparticle size. The kit also includes protocols for both hand-operated and syringe pump-operated CIJ-based synthesis and is used with the **NanoFabTx™ NanoFlash CIJ instrument**, including the CIJ device, fittings, and tubing required to get started with the CIJ-based synthesis process.

NanoFabTx™ NanoFlash PEG 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 particles.
Storage and stability: Store kit at -20°C. Protect from light. Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials required, but not supplied:

Catalog Number	Quantity
E7023	Ethanol
Z693472	Magnetic Stirrer
Z266337	Stir bars (40 mm x 8 mm)
27024	Glass vials, clear glass (4 ml capacity)
27172-U	Glass vials, clear glass (22 ml capacity)
SLFH025	Syringe filters 0.45µm (for filtering non-aqueous solutions like polymer/drug solutions)
SLHAR33SS	Syringe filters 0.45µm (for filtering aqueous solutions like stabilizer solution)
D9777	Dialysis membrane (12-14kDa cut off)
09978	Ammonium sulfate, BioUltra, ≥99.0%
	Deionized water
D1515	doxorubicin hydrochloride or hydrophilic drug of choice
	Syringes compatible with syringe pumps required (recommended with Hamilton® GASTIGHT® syringes)

Materials required for syringe pump method, but not supplied:

Item description
Syringe pump (protocol requires one pump)-recommended with Harvard Apparatus – PHD Ultra pumps

Lipid solution preparation:

- Prepare the desired formulation provided in table 1 (e.g., add 10 mg of the lipid to 5 ml of Ethanol to prepare a 0.2 wt/v% of lipid solution).
  Note: *Concentrations provided in table 1 are optimized for the model drug, doxorubicin hydrochloride. Utilizing a different drug molecule and alternate concentrations may result in variations in nanoparticle properties, including size and encapsulation efficiency.*
- Gently vortex the solution for 1-2 minutes to fully dissolve the lipid. The final solution should be a clear and transparent mixture.
- Note: If necessary, heating the lipid solution can aid in dissolving the lipid mix.
- Before use, filter the solution through a 0.45 µm syringe filter to remove any particulate matter. Please note that the lipid solution should not be stored for longer than 24 hours.

Drug solution preparation:

- Prepare ammonium sulfate (AS) buffer (240mM, pH 5.4) by dissolving 31.71g of ammonium sulfate salt in 1 liter of deionized water. The pH is natively 5.4, additional pH adjustment is not required.
- Prepare the desired formulation provided in table 1.(e.g. for drug to lipid weight ratio of 1:10 for 0.2 wt/v% formulation, prepare a 0.02 wt/v% drug solution)
- Filter it through a 0.45 µm syringe filter before use.

Procedure 1- Hand-operation with subsequent dilution to synthesize drug-encapsulated PEGylated liposomes

Set up/assemble CIJ instrument:

Prime CIJ instrument:

- Fill two syringes with THF or solvent of interest and load them on CIJ
- Use the metal plate to connect two syringes and push the syringes rapidly.
- Collect the solvent at the outlet in a waste container and repeat the process three times.
- Note: Priming purges gases from the fluid pathways and serves as a check of chemical compatibility for all wetted parts of the system. In addition, priming reduces or prevents precipitation of polymers and drug inside the system. Precipitation of polymers and drug can obstruct the mixer channels and tubing.

Drug encapsulated PEG lipid mix nanoparticle synthesis:

- Once the lipid solution has been prepared, transfer the desired volume of the solution into a syringe.
- In a separate syringe, transfer an equal volume of the drug solution.
- Attach both syringes containing the lipid and drug solution onto the CIJ device.
- Transfer 8 times the volume of lipid solution of the DPBS into a beaker.
- Use the metal plate to connect two syringes to ensure simultaneous actuation and push the syringes in a rapid motion.
- Collect the nanosuspension at the outlet tube into the beaker of DPBS.

  Note: Discard the first and last drops of the outlet suspension to obtain a more monodispersed particle population.
- Clean CIJj instrument:
- Clean the CIJ instrument by filling two syringes with THF or your desired solvent and loading them onto the CIJ.
- Connect the two syringes with the metal plate and rapidly push them to ensure simultaneous actuation.
- Collect the solvent at the outlet into a waste container and repeat the process three times.
- Note: Improper cleaning can result in blockage in the mixer channels and tubing.
- Remove excess solvent and unencapsulated drug:
- Transfer the nanoparticle suspension to a 12-14kDa cut-off cellulose membrane.
- Dialyze against 4L deionized water for 4-6 h at room temperature, change the water after 30 minutes and 1 hour.

Procedure 2- Syringe pump-operation with subsequent dilution to synthesize drug-encapsulated PEGylated liposomes

Set up CIJ instrument:

Prime CIJ instrument:

- Fill two syringes with THF or solvent of interest and load the syringes on the syringe pump.
- Adjust the flow rate at 30 ml/min.
- Run the syringe pump and collect the solvent at the outlet in a waste container and repeat the process three times.

  Note: Priming purges gases from the fluid pathways and serves as a check of chemical compatibility for all wetted parts of the system. In addition, priming reduces or prevents precipitation of polymers and drug inside the system.

  Precipitation of polymers and drug can block the mixer channels and tubing.

PEG lipid mix nanoparticle synthesis:

- Once the lipid solution has been prepared, transfer the desired volume of the solution into a syringe.
- In a separate syringe, transfer an equal volume of the prepared drug solution.
- Attach both syringes containing the lipid and drug solution onto the CIJ device.
- Transfer 8 times the volume of lipid solution of DPBS into a beaker.
- Load the syringes on the syringe pump.
- Adjust the flow rate to 10 ml/min.

  Note: The particle size can be adjusted by varying the flow rate and *concentration of the lipid and drug. Typically, a decrease in both the concentration of the lipid and drug and an increase the flow rate results in the formation of smaller nanoparticles.*
- Run the syringe pump and collect the nanosuspension at the outlet into the beaker of DPBS.

Note: To obtain more monodispersed particles, discard the first and last drops of the outlet suspension.

- Clean cij instrument:
- Clean the CIJ instrument by filling two syringes with THF or your desired solvent and loading them onto the CIJ.
- Adjust the flow rate at 30 ml/min.
- Run the syringe pump and collect the solvent at the outlet in a waste container and repeat the process three times.

Note: Improper cleaning can result in blockage in the mixer channels and tubing.

Table 1. List of formulation suggested to be prepared

Formulation	Initial polymer concentration (wt/v %)	Lipid to drug weight ratio
0.2 wt% lipid	0.2	0
0.2 wt% lipid-10	0.2	10
0.2 wt% lipid -20	0.2	20

Physiochemical characterization of nanoparticles:

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

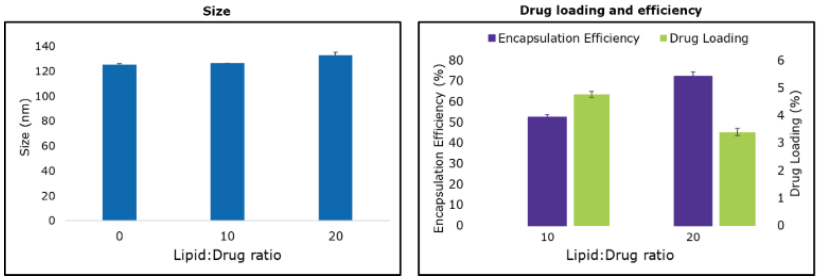

Figure 1. Example data of PEGylated liposome size, encapsulation efficiency and drug loading versus lipid: drug ratio using a model small molecule drug. Working flow rate range: 10-45 ml/min, PDI <0.2.