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liposome-kit/txtl-liposome_water-in-oil.md at master · BuildACell/liposome-kit

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murrayrm Added comment about using an inverted microscope

1 contributor

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TX-TL Liposome Using Water-in-Oil Emulsion

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Overview and Materials

This protocol describes how to create liposomes that contain TX-TL inside of a lipid-based container. The liposomes created by these protocols are between 1 and 10 nm in diameter and should be usable with an cell-free protein expression system.

The preparation of liposomes using w/o emulsions as template requires four steps:

Step 0: Deposition of a thin lipid film in a glass vial

Materials:

- Glass vials (any will do, but we use 2mL Fisherbrand Class B Clear Glass Threaded vials: cat #: 03-339-21A)
- POPC in chloroform: <https://avantilipids.com/product/850457>
- Liss-Rhod-PE: <https://avantilipids.com/product/810150>
- Cholesterol in chloroform (25 mg/ml): <https://avantilipids.com/product/700000>
- Glass syringes of various sizes (10 μL, 50 μL, 250 μL, and 1 mL) are suggested ex. Hamilton gastight cat #: 14-815-238, but any will do for use with lipids and chloroform <https://www.hamiltoncompany.com/products/syringes-and-needles/general-syringes/gastight-syringes>

Step 1: Preparation of the oil containing lipids

Materials:

- Mineral oil: <https://us.vwr.com/store/product/7422728/mineral-oil-light-white-high-purity-grade>

Step 2: Self-assembly of the Liposomes by centrifugation

Materials:

- 20 μM of HPTS stock solution (<https://www.thermofisher.com/order/catalog/product/H348>)
- 100 mM HEPES, 200 mM glucose pH 8 ✓
- 100 mM HEPES + 250 mM glucose, pH 8 ✓

https://github.com/BuildACell/liposome-kit/blob/master/txtl-liposome_water-in-oil.md

- all stock have been made 50mL each
- Liss-Rod opened and moved to vial and stored at -20°C
- cholesterol was made at 50 mg/mL
- Internal solution also made, needs to be diluted when used!

HEPES 238.3 g/mol
100 mol
238.3 g

$$\frac{100 \text{ mol}}{1000 \text{ L}} \times \frac{\text{SOL}}{1000} \times \frac{\text{mol}}{238.3 \text{ g}}$$

0.005 mol × 238.3 g/mol = 1.1915 g HEPES

Glucose 180.16 g/mol
 $\frac{200 \text{ mol}}{1000 \text{ L}} \times \frac{50 \text{ L}}{1000} \times 180.16 \text{ g/mol} = 1.8016 \text{ g}$

Sucrose 342.37 g/mol
200mM
 $\Rightarrow 34.23 \text{ g}$

250mM → 2.252 g

HPTS 524.37
 $\frac{2 \text{ mol}}{1000 \text{ L}} \times \frac{1 \text{ mL}}{1000} \times \frac{524.37 \text{ g}}{\text{mol}} = 1.048 \text{ mg for 2mM HPTS}$
1:1000 dilution for 2uM

Math to make all stock solutions.
pH was measured to be 7.97 in all cases.

Step 0: Deposition of a thin lipid film in a glass vials

The first step in the protocol is to generate the lipid film required to form the lipid-in-mineral oil solution. Makes 6 samples with accurate measurement. Each vial will have ~ 15 mg of lipid with 0.1 mol % of 18:1 Lyss-Rho-PE.

1. Create lipid master mix in a glass beaker. One of two methods may be used:

- o POPC/Lyss-Rho-PE

a. Add 4 mL (100 mg) of POPC in chloroform (25 mg/ml)

b. Add 200 uL (0.2 mg) of Lyss-Rho-PE in chloroform (1 mg/mL)

c. Swirl gently until all POPC is dissolved and color is homogeneous.

- o POPC/Cholesterol/Lyss-Rho-PE ✓

a. Add 2.668 mL (66.7 mg) of POPC in chloroform (25 mg/ml)

666 b. Add 1.332 mL (33.3 mg) of Cholesterol in chloroform (25 mg/ml) 50

c. Add 200 uL (0.2 mg) of Lyss-Rho-PE in chloroform (1 mg/mL)

d. Swirl gently until all POPC is dissolved and color is homogeneous.

2. Aliquot 700 uL of POPC/Cholesterol/Lyss-Rho-PE Chloroform solution into 2 mL glass vials (6 vials total) ✓

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3. Place uncapped vials in fume hood, loosely covered with aluminum foil and allow to evacuate overnight in fume hood ✓ (~6-8 hours).

- o Note: The purpose of the aluminum foil cover is to protect particles from contaminating the POPC/Cholesterol/Lyss-Rho-PE as it evaporates.

4. Move vials to vacuum chamber and vacuum for an additional 2 hours.

5. Store in -20 degC.

Note: Remaining POPC in chloroform, cholesterol, and Lyss-Rho-PE in chloroform can be stored at -20 degC in the glass vial with PTFE caps and sealed parafilm.

- 1mL Syringe was rinsed with Chloroform to clean, after each use
 - since all the measurables will be in a chloroform solution
- All six vials were wrapped w/ aluminum foil
 - protect the lipid film from light

* would be nice to have a rotating stage such that the lipid film would be uniform and even on the bottom of the vial

*no images were taken during the process of Step 0.

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Images of the 6 Vials [end of Step 0]



*Some images not
in focus