Nucleus Protocols

Encapsulation: Phase Transfer

Hazardous Materials

Chloroform - Irritant, possible carcinogen. Work in fume hood and use gloves.

1. Prepare Lipids in Mineral Oil

- ☐ Add 1 mL of mineral oil to the glass vial using a 1 mL pipette.
- ☐ Add the lipids in the above table to the glass vial on top of the mineral oil using the appropriate glass syringe.
- ☐ Briefly vortex the lipid-oil mixture for 5 s to mix.
- ☐ Evaporate the chloroform from the lipid–oil mixture:
    - ☐ Place glass vial in a 55 °C dry bath in a fume hood.
    - ☐ Shield with aluminum foil to protect from light.
    - ☐ Evaporate uncovered for 4 h.
- ☐ Meanwhile, clean your glass syringe.
    - ☐ Add 4 mL of chloroform to a glass bottle using a glass 10 mL serological pipette.
    - ☐ Rinse your syringe with that chloroform 5 times.
    - ☐ Dry the syringes with the plunger removed inside the fume hood for 3 h to 4 h to allow remaining chloroform to evaporate.
- ☐ Cool the lipid-oil mixture to room temperature (between 10 min and 15 min). Your lipid-oil mixture is now ready for use.
- ☐ Return the plungers to the syringes and store them in their designated location.
- ☐ Dispose of chloroform waste following applicable chemical safety guidelines.

2. Assemble Outer Solutions

- ☐ Prepare 1.5 mL microcentrifuge tubes labeled with the appropriate reaction.
- ☐ Prepare outer solution by mixing glucose stock solution and water to reach your target osmolarity:

Component	Outer Solution (µL)
Glucose (2 M)	570
Water	430
Total	1000

3. Encapsulate Inner Solution

- ☐ Allow lipid-oil mixture to come to room temperature as you perform these next steps.
- ☐ For each liposome encapsulation, set up a 1.5 mL tube rack with two 1.5 mL micro-centrifuge tubes. Number the tubes according to the number of reactions assembled. Label the two tubes for each reaction:
    - ☐ **T**—transfer
    - ☐ **L**—liposomes
- ☐ Add 300 µL of the appropriate glucose outer solution to each of the tubes labeled **T**. Vortex thoroughly and spin down.

- ☐ Assemble at least 30 µL of your inner solution per reaction (see Assemble Base Cytosol for an example).
- ☐ Once at room temperature, add 150 µL of the lipid-oil mixture on top of each assembled inner solution reaction.
- ☐ Emulsify the lipid-oil and inner solution reaction by running the tube along a row of empty slots on the 1.5 mL tube rack. Drag your tube across empty slots at least 50 times until you form a stable emulsion with a homogeneous milky appearance.
- ☐ Immediately layer each emulsion on top of the outer solution by slowly pipetting down the side of the corresponding **T** tube.
- ☐ Centrifuge **T** tubes at 9000 g for 10 min at room temperature to pellet the liposomes.
- ☐ Extract the liposomes from each **T** tube:
    - ☐ Gently remove the oil layer and lipid debris from the top of each **T** tube with a 1000 µL pipette.
    - ☐ Gently resuspend the pellet by mixing by pipette 10-15 times.
    - ☐ Extract liposomes by pipetting 50 µL to 100 µL of pellet and outer solution from **T** and transfer liposome sample to the respective liposome tube **L**.
- ☐ Hold liposomes on ice until you are prepared to begin measurement.
- ☐ Transfer 5 µL liposomes and 20 µL of corresponding outer solution onto a 384-well glass bottom plate (5x dilution). If the density of liposomes appears too high under the microscope, dilute them with outer solution to facilitate data analysis.
- ☐ Observe liposomes by fluorescence microscopy.