Echelon Biosciences Inc.

Preparation of Multilamellar Liposomes (LMV)

This protocol is recommended as a general guide for preparation of liposomes from lyophilized lipids. Additional considerations and common troubleshooting points are addresssed in our Liposome FAQ.

All solvent solutions should be brought to room temperature before applying to lipids. Please read the entire protocol before beginning.

Procedure :

1. Dissolve lipids in chloroform and combine in the appropriate ratios.
2. Evaporate Chloroform from lipid mixtures using a dry nitrogen stream or vaccum (eg. SpeedVac). Dried lipid mixture can be stored for up to 6 months at –20 °C.
3. Re-suspend the lipid mixture in cyclohexane. If the mixture is not completely soluble in the cyclohexane, add a small amount of ethanol (1-2 % of the cyclohexane volume). Do not use too much ethanol, as the solution will not freeze with excessive ethanol present.
4. Freeze cyclohexane solution using dry ice or place at –20 °C for 30 min to 1 hour depending on volume of cyclohexane used.
5. Quickly place the frozen mixture on a high vacuum system (lyophilization system).
    - Generally, "house vacuum" systems are not strong enough for this process. The sample should remain frozen until it is completely dry; if the sample begins to thaw, either the vacuum is not strong enough or there is too much ethanol present. A thawed sample will not produce a white powder and it may bump or foam out of the vial.
    - Leave the sample on the vacuum system until the sample is completely dry. The vial should not feel cold to the touch or smell of cyclohexane when removed from the vacuum.
    - This produces a dry, white powder, which readily suspends in water and dried lipid mixture can be stored for up to 6 months at –20 °C.
6. Suspend the lipid mixture in the aqueous buffer (buffer temperature should be above the phase transition of the lipid. See Liposome Q&A) and allow the mixture to hydrate above the transition temperature of the lipid for 30-60 minutes (vortexing occasionally).
    - For encapsulating water-soluble compounds in the liposome the same protocol is followed except compound is dissolved in the aqueous buffer before reconstituting the dry lipid. The external compound (not encapsulated) can be removed by gel filtration.

Note: An alternative method is to skip steps 3-5 and resuspend the lipid film produced by evaporating the chloroform into the appropriate aqueous buffer. See step 6.

References

1. Frank Szoka, Jr. & Demetrios Papahadjopoulos, (1980), "Comparative Properties and Methods of Preparation of Lipid Vesicles (Liposomes)", Ann. Rev. Biophys. Bioeng., 9:467-508.

Echelon Biosciences Inc.
Ph: 866-588-0455
Fax: 801-588-0497
Echelon-inc.com