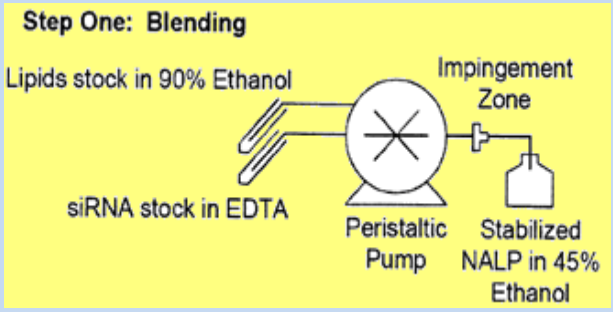
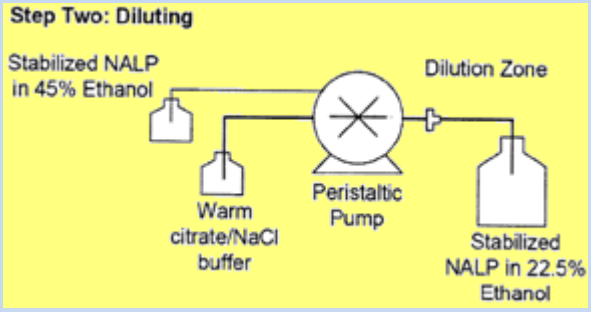
Protocol for Stable Nucleic Acid-lipid Particle (SNALP) Preparation





Reagents & Materials:

1. Active agent: (eg: siRNA, shRNA, aiRNA, miRNA)
2. Cationic lipid: (eg: Dlin-K-C2-DMA, Dlin-K-DMA, Dlin-M-C3-DMA)
3. Neutral lipids: (eg: DPPC, Cholesterol, DSPC, DSPE, DMPC,PEG-DSPE)
4. A Lipid conjugate: (eg: C16 Ceramide-PEG2000, PEG2000-C-DMA, PEG-DAA. PEG-DAG)
5. Antioxidant/diluted solution: (1:1 with 20mM citrate buffer, 300mM NaCl, pH6.0).

Procedure:

1. The lipids are initially dissolved in an ethanol enviroment of about 80%~90%.
2. The lipid solution is diluted stepwise by mixing with an aqueous solution of siRNA resulting in the formation of vesicles at an ethanol concentration of about 20.0%~55%.
3. By mixing the aqueous solution with the organic lipid solution, the organic lipid solution undergoes a continuous, sequential stepwise dilution to produce a liposome. Further, lipid vesicles such as SNALP can be further stabilized by an additional stepwise dilution of the vesicles to an ethanol concentration of less than or equal to about 25%,preferabley between about 19~25%.
4. The additional sequential dilution is performed substantially immediately after formation of the liposomes. For example, it is advantageous that less than 1 minute elapse between liposome solution formation and dilution, more advantageously less than 10 seconds,and even more advantageously less than a second or two.
5. The free therapeutic agent can be removed from the formulation through anion exchange chromatography.
6. The liposome solution can be concentrated 10-20 fold through ultrafiltration.
7. The concentrated formulation is further diafiltrated against about 10 volumes of aqueous solutin to remove ethanol, which is less than about 1%.
8. After the ethanol has been removed, the aqueous solution is then replaced by dialfiltration against another fuffer (eg: 15 mM NaCl with 10 mM Hepes, pH7.4).
9. The liposome solution will be sterilized by passing through a sterilizing membrane with a particle discrimination size of about 0.2 microns.