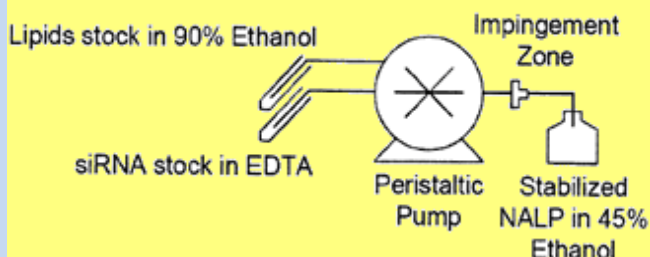
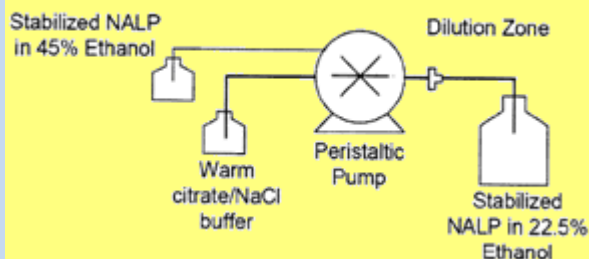


Protocol for Stable Nucleic Acid-lipid Particle (SNALP) Preparation

Step One: Blending



Step Two: Diluting



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 environment 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%,preferably 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 solution 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 buffer (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.