

DOTAP Chloride

Catalog Number: 14475

DESCRIPTION

DOTAP Chloride (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride) is a widely used cationic liposome-forming compound for *in vitro* and *in vivo* transfection of DNA, RNA and other negatively charged molecules. DOTAP encapsulates the nucleic acids to form a stable complex spontaneously by means of electrostatic interaction, facilitates cell attachment, internalization by endocytosis as well as endosomal escape by proton sponge. It has been used in the formation of Lipid Nanoparticles (LNPs) or Nanostructured Lipid carriers (NLCs) in the development of mRNA vaccines.

SPECIFICATIONS

CAS Number	132172-61-3
Purity	>99%
Molecular Weight	698.54
Appearance	solid
Storage	-20°C under argon gas
Linear Formula	C42H80ClNO4
Synonym(s)	N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride

LIPOSOME SYNTHESIS

Materials

- DOTAP
- Purified H₂O or Phosphate-buffered saline (PBS) at pH 7.4
- Chloroform (or other organic solvent)

Equipment

- 5 mL Glass beaker
- Glass round bottom flask
- Rotary evaporator
- Water bath sonicator
- Vortex Mixer
- Sterile polystyrene storage tube
- Pipette with appropriate tips
- Calibrated scale
- Laminar flow hood with vacuum
- Extruder with appropriately sized polycarbonate membrane
- Block heater



Prepare Stock Solution

1. Remove DOTAP from freezer (-20 °C) and thaw to room temperature.
2. Weigh 25 mg of DOTAP into glass vial.
3. Add 2.5 mL of chloroform to the glass vial; agitate until dissolved.
4. (Optional: Incubate at 37 °C for 10 minutes to facilitate homogenization).
5. (Optional: Store stock solution at -20 °C under nitrogen or argon).

Preparation of Lipid Film

1. To create lipid film, place stock solution in round bottom flask and use a rotary evaporator to remove the organic solvent.
2. Evaporate chloroform with dry nitrogen flow in fume hood.
3. To remove any residual organic solvent and ensure the lipid film is fully dry, place the vial in a vacuum pump at a temperature of 45-60 C overnight.

Rehydration

1. Add 1 mL filtered H₂O or PBS to lipid film.
 - a. Warm to 37 °C for 10-30 minutes for homogenization; gently vortex as needed.
 - b. If dry lipid film remains on flask, sonicate for 15 s in a water bath sonicator.

Extrusion

1. Prepare extruder:
 - a. Assemble the extruder with 400 nm polycarbonate membrane and lubricate with 1 mL filtered H₂O or PBS, then discard solvent.
2. Extrude 1 mL of solution by passing through the extruder 5 times.
 - a. Repeat with smaller polycarbonate membranes (200 nm, 100, nm, and 50 nm) if smaller sized liposomes are desired.
3. Store suspension in a glass vial under nitrogen or argon at 4 °C until use, up to one month.

