

## Preparation of liposomes

### Materials

1. Soybean azolectin (Fulka, Buchs, Switzerland)
2. Chloroform and acetone (Nacalai tesque, Kyoto, Japan)
3. Sonicator (Digital Sonifire model 250 D, 200 W, 20 kHz)(Branson, Danbury, CT)
4. Mini extruder (Avanti Polar Lipids, Alabaster, AL)

### Methods

1. Dissolve 10 g of azolectin in 30 ml of chloroform.
2. Add 180 ml of ice-cooled acetone to the solution and stir the suspension on a magnetic stirrer for 2 h at room temperature.
3. Turn off the stirrer and allow the solution to stand overnight at 4 °C in order to precipitate phospholipids.

4. Discard the supernatant as much as possible and dry the pellet completely under flow of nitrogen gas.
5. Store the dried phospholipid mixture at -20 °C until use.
6. Prepare the liposome suspension at 10~100 mg/ml in Milli-Q water.
7. Sonicate the liposome suspension (15% amplitude and 30% duty cycle) on ice until the appearance changes from milky to nearly transparent.
8. Prepare uniform-size liposomes by extrusion through a mini extruder.  
  
Assemble the extruder according to manufacture's instruction. Pass the liposome suspension 11 times through 100~400 nm membrane.