

Ultra-centrifugation

Aim

Ultracentrifugation is used for purification of phage solutions. A purification and further concentration of the particles is carried out by Sucrose step gradient ultracentrifugation.

Materials

- Ultracentrifuge
 - Swinging-bucket Rotor
 - Ultracentrifugation tube (open top)
- Sucrose solutions with different concentrations
- Phage solution
- Cannula + sterile syringe

Procedure

Preparation of samples

- Preparation of sucrose solutions of different concentrations (20 % (w/v) till 70 % (w/v) in 5 % steps)
- Fill sucrose gradient into ultracentrifugation tube by adding equal volumes of each sucrose solution starting with the highest concentration going to the lowest
- Freeze at -80 °C for 5 min
- Store at -4 °C O/N
- Thaw the samples for 24 h on site (avoid movement after thawing)
- Fill sample to 2 mL with phage solution (weight both samples to same weight)

Ultra-Centrifugation

- Centrifugation at 22.000 rpm for 1 h and 22 °C (+ swing out without stop for 1 h)

Extraction of purified phage solution

- Extract separated phase containing the phages with cannula and disposable syringe in front of a dark background
- Determine the phage titer ([Agar overlay plaque assay](#))