

Ultracentrifugation of Viral Particles

Hint: keep in mind that you are handling highly infectious material! Avoid aerosol or contamination at any cost. Carefully follow your lab's safety instructions.

1. Precool the ultracentrifuge to 4°C.
2. Centrifuge 20-30ml of freshly harvested supernatant containing viral particles at approx 2500xG for 5min to pellet remaining debris.
3. (optional, recommended) filter supernatant through a .45um syringe filter
4. Fill 4ml of 20% Sucrose dilution into the ultracentrifuge tube.
5. Carefully overlay 20-30ml of virus supernatant, **do not mix with Sucrose cushion** (slightly tilting the tube may help).
6. Make sure you have two tubes with exactly the same weight (!) Put the closed tubes into the ultracentrifugation rotor and firmly close the rotor lid (vacuum grease may be needed).
7. Centrifuge with 50.000xG at 4°C for 2hrs.
8. Decant supernatant and resuspend virus pellet in a small volume of PBS+1% FCS or serum free medium (0.1-1ml)

Material: 50ml falcon tubes, ultracentrifuge tubes with lid, pipettes. optional: 0.45um syringe filters, 50ml syringes with luer lock.

Reagents: 20% Sucrose (=Saccharose; in aqua dest, filtered sterile), PBS (+1% FCS).