VIRUS PURIFICATION THROUGH 25% SUCROSE

MATERIALS

1. Ultracentrifuge
2. ultracentrifugation tubes
3. 10cm sterile petri dishes
4. 50mM Sodium Buffer
    - 50 mM Sodium Phosphate Buffer pH 7.4
    - Prepare 100 ml of 1 M of Na_2HPO_4 (FW 141.96) **solution A**
    - Prepare 100 ml of 1 M of NaH_2PO_4 (FW 120.0) **solution B**
    - Take 7.74 ml of solution A and 2.26 ml of solution B **solution C**
    - Take 5 ml of solution C and dilute in 95 ml distilled H_2O $\rightarrow$ **50mM Sodium Phosphate Buffer**
5. 25% sucrose solution
    - Weigh 25 g of sucrose and make up to 100 g with 50mM Sodium Phosphate Buffer pH 7.4
    - Place 200 µl tip onto balance & rezero
    - Transfer this tip onto pipette & collect 100 µl of sucrose solution
    - Clean drops off the outside of the tip with a tissue
    - Remove tip from the pipette and place on balance
    - Multiply the result by 10 to calculate g/ml concentration: Density should be 1.12g/ml
    - Filter sterilize in the MSC hood
    - Use Nalgene 100 ml filters
6. 70% EtOH
7. 50ml Syringes
8. 0.45µm sterile Filter
9. balance
10. Media for virus storage
11. Cryovials

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METHODS

1. Turn on ultracentrifuge, place rotor inside, close lid, set temperature to 4°C and start vacuum
2. Cool buckets in refrigerator.
3. Sterilise ultracentrifuge tubes
    - Fill each tube with 70% ethanol & leave in MSC hood for 2-5 minutes
    - Empty tubes (? recycle ethanol) & leave in hood to dry for 10 minutes
4. Harvest virus by low speed spin (400g 5 min) & 0.45µ filtration
5. Transfer virus suspension into tubes (maximum 25 ml)
6. Underlay virus suspension with 5 ml 25% sucrose solution
    - avoid bubbles & touching side or bottom of tube
7. Insert filled tubes into buckets
    - Use matched buckets (1&4, 2&5, 3&6)
8. Balance buckets. For each matched pair:
    - Weigh matched buckets
    - Re-zero scales with heavier bucket
    - Re-weigh lighter bucket and bring up to zero with sterile PBSA
9. Tightly close bucket lids **(ensure all have the same orientation)**
10. Stop vacuum. Open centrifuge and place buckets into rotor GENTLY.
11. Close centrifuge and start vacuum immediately
12. Adjust ultracentrifuge settings with arrows and pressing enter, on screen:
    - Speed: 23 000 rpm / Time: 02:00 hrs / Temperature: 4°C / Acceleration: 9 / Deceleration: 4
13. Press START
14. Prepare media for resuspending virus & labelled cryovials
15. After centrifugation transfer buckets to MSC hood
16. Extract tubes with tweezers & discard supernatant into Trigene bath
17. Invert tubes onto sterile plastic dish to allow remaining liquid to run out
18. Re-suspend viral pellet and prepare aliquots for liquid nitrogen storage.