

## **FV3 purification protocol**

(Adopted from Ann Casey, Federoff/ Bowers lab)

### **Notes:**

- Use BHK21 cells infected at >90% confluency with MOI~1 stored at 30°C .
  - Incubate with FV3 for 3-4 days, checking daily for CPE. Approx. 3 days post infection CPE should be close to 90%. Harvesting should not happen at CPE >99% as virus may inactivate in dead cells.
  - Pre-incubate ultracentrifuge buckets in 4°C before use.
1. Freeze and thaw infected cells 3x, vigorously vortex slurry, do not allow full thawing
  2. Centrifuge at 2500rpm(1400xg), 10min, 4°C
  3. Recuperate supernatant (S1) store on ice, and resuspend pellet in no more than 20ml M-SF (serum free media)
  4. Sonicate pellet 30sec, pause, 30sec, add ice frequently (keep cold).
  5. Centrifuge 10min at 2500rpm (1400xg), 4°C
  6. Recuperate sup (S2), and combine with (S1)
  7. Store on ice until ready to load or store at 80°C
  8. Sterilize ultracentrifuge tubes with 70%EtOH 2x and UV light for 20min
  9. Let tubes air dry completely under the hood before use.
  10. Add 5-7 ml cold 1X DPBS to the ultracentrifuge tubes
  11. Underlay with 7-10ml 30% Sucrose in DPBS, slowly dispensing it at the bottom of the tube
  12. Spin viral supernatant 10min, 2500rpm(1400 x g), 4°C
  13. Overlay with viral supernatant (~ 20ml) to ~ 1cm from the top
  14. Overlay with DPBS to ~1/4 cm from the top
  15. Ultracentrifuge 1hr, 24,000rpm, 4°C
  16. Add ~100ul cold 1X DPBS/tube and let sit ON. Pellet should look iridescent
  17. Resuspend and ensure pellet solution
  18. Store at -80C