

## Virus Purification

- pellet cells at 3,000 rpm for 25 mins 4°C (6 liters)
- pellet virus at 9,000 rpm for 2 hours 4°C
- Resuspend pellet immediately in 0.5 ml PO4 buffer to a final volume 2.5 ml and transferred to a Tml douncer
- PMSF added 100 µg/ml to prevent breakdown of virus by cell-associated proteases
- Virus pellet disrupted by 3 strokes of a tight douncer.
- Suspension clarified by centrifugation 5,000 rpm for 15 mins in SS-34 rotor and dialyzed again 0.5 ml PO4 buffer 1 hour
- Crude Virus layered onto a 36 ml 5-30% (wt/wt) dextran gradient and centrifuged at 20,500 rpm 1 hr  
(76,000 x g)
- Visible virus band removed by side puncture w needle & syringe
- Diluted 1:1 w 0.5 ml PO4 buffer and layered onto 34 ml 10-30% dextran
- Centrifuge 22,000 rpm 1 hr SW27  
(90,000 x g)
- Virus removed by side puncture then diluted in PO4 buffer and centrifuged at 25,000 rpm for 1.5 hrs.  
(116,000 x g)
- Virus pellet resuspended in 0.05 ml PO4 buffer at frozen at -20°C