Production of Viral Stock (JWK Method)

1. Seed $3x10^7$ SF-9 Cells into each $225cm^2$ Flasks, and put at 27^oC for 60 minutes.

2. Pour off medium and add 20mL of virus in Grace's medium (Multiplicity of Infection, or MOI = 1-2; if the titer of the primary infecting stock is unknown, use about a 1:350 final dilution, i.e. 200uL of infecting stock per 20mL of Grace's. After step 3, this will give a 1:350 dilution). Return to 27^oC for 1 - 2 hours.

3. Add 50mL of TMN/FH medium, loosen caps, and return to 27^oC for about 7 days.

4. Harvest virus solution by spinning at 1000 rpm for 10 minutes to remove cellular debris. Maintain sterility. Store viral stocks at 4^oC, in the dark.

5. Using this method, titers of $>5x10^8$IU/mL have been obtained. If a titer is necessary, it may be determined by infecting Hi-5 cells with a 10-fold serial dilution, and observing the infectivity.

TMN/FH Medium

1000mL Grace's Medium
20mL 50X Lactalbumin Hydrolysate
20mL 50X Yeastolate
10mL 100X Pen-Strep-Amphotericin
10mL 10% Fluronic F68 (for spinner culture only)
100mL Fetal Bovine Serum

Counting Cells with the Coulter Counter

1. Measure 20mL of counting fluid into a truncated 50mL conical tube

2. Add the aliquot of cells you are counting and mix

3. If counting primary cells, use Zapoglobin II. 6 drops for lymphocytes and 8 drops for macrophages, etc. Mix, and place in the counter. Select setting for Zap or no Zap.

4. Zero counter by turning the upper stopcock to vertical position. Count cells by turning the upper stopcock to the horizontal position. When the number is constant, the count is finished.

5. Calculation of cells/mL: (#cells x 40[a])/mL added

[a] 40 is the constant derived from diluting into 20mL and the 0.5mL which machine counts