**Production of rKSHV.219 and determination of infection units (IU)**

The production of infectious rKSHV.219 is carried out by propagating latent virus in Vero cells, and then inducing lytic replication.

For the generation of rKSHV.219 stocks, Vero cells (80-90 % confluent) were infected with Back50 for 2 hours in 4 ml of serum-free DMEM.

* First, determine how much of the Back50 stock you will need to infect Vero cells with an MOI of 0.5 to 1. I infected Vero cells with an MOI of 0.75. There are usually about 4 x 106 cells in a T 75 flask.

**Example:**

Desired MOI =0.75

Amount of cells in T75= 4 x 106 cells

Back50 titer (stock at 4 degrees)= 375x 106  pfu/ml

(0.75)x (4 x 106 )= **3x106 (amount of Back50 from stock). Then,** How much from the stock do I need to get **3x106 :**

**3x106 /** 375x 106 pfu/ml = 0.008 ml = 8ul of Back50 stock. Make a 1:10 dilution of the Back50 stock, to take 80ul rather than 8ul.

* Be sure that Vero cells are 80-90% confluent. If that is the case, then they are ready to be infected. Remove all the medium from the cells (Previous to infection, Vero.219 cells were maintained in DMEM + 10% FBS + 1% Penicillin /Streptomycin + 5ug/ml of puromycin). For this step, I had 3 flasks with Vero.219 cells for infection. Therefore, I prepared an INFECTION MASTER MIX as follow:

15 ml of DMEM + 5ug /ml of puromycin (don’t add serum in this part)

240 ul of the 1:10 diluted Back50.

Add 4 ml of the master mix containing the inoculum (Back50) to each flask, and incubate them for 2 hours in the incubator at 37 degrees.

* After 2 hours, remove the medium from the flasks, and wash the cells once with PBS (wash them as gently as possible). Add to each flask 12 ml of fresh complete medium with 1.25 mM of sodium butyrate. Complete medium will contain: DMEM + 10% FBS + 1% Penicillin /Streptomycin + 5ug/ml of puromycin + 1.25 mM of sodium butyrate. Put the flasks back to the 37o C incubator.
* Twenty to thirty hours later, **remove the medium with sodium butyrate** and add to each flask 12 ml of fresh complete medium **without puromycin**: DMEM + 10% FBS + 1% Penicillin /Streptomycin. Put the flasks back to the 37o C incubator.
* Next day collect the virus.