Production of protein in Hi-5 cells in Spinner Flasks

The final concentration of Hi-5 cells in the infected culture should be $5x10^5$ c/mL. Grow your Hi-5 cells to between $5x10^5$ and $2x10^6$c/mL. It uses less medium to grow to higher densities.

Using Corning 250mL conical centrifuge tubes, spin the cells at 1000 rpm in the IEC centrifuge. Use the carriers with the rigid white plastic inserts. The tubes may be balanced by putting the same volume in opposite carriers and using opposite carriers with identical identification numbers.

Prepare Grace's Medium + 1x F-68. For each Liter of final infected volume, add 300mL of the Grace's + F-68 to a 1L spinner. Pour off the culture supernatant from the pelleted Hi-5 cells, and resuspend each pellet in 10 mL of the Grace's + F-68 which is in the spinner. Place all the Hi-5 cells into the spinner.

Add your virus stock to the spinner, usually a 1:100 dilution of the virus, based on the final volume, e.g. 10mL virus stock/L final volume. Put the spinner at 27^oC on a stirrer, at about speed 2. Infect for 1 - 2 hours.

Pour the amount of TMN/FH + F-68 medium you will need into a sterile container, (or HyQCCM3, if using serum-free conditions). Do not pour from your medium bottle directly into a virus culture, to avoid cross-contamination and infection of your stock cells. For each liter, add 750mL of medium, and return the spinner to 27^oC. Stir at speed 3.5.

Harvest the Supernatant on Day 6. Day 0 = day the cells were infected.