

### **PBL/PBMCs culture and infection**

Medium: 500 ml RPMI 1640  
100 ml heat-inactivated FBS (20%)  
5 ml L-glutamine (1%)  
50 ml IL-2 (10%)  
1 ml gentamicin (0.2%)

1. Resuspend PBLs/PBMCs ( $80 \times 10^6$  cells) in medium - 8 ml final volume of cells + RPMI (final conc. =  $10 \times 10^6$  cells/ml) and put in a T-75 flask.
2. Add 40  $\mu$ l PHA (final conc. = 5  $\mu$ g/ml) to the flask. Incubate at 37 °C for 30 min.
3. Add 32 ml medium to the cells and incubate overnight \ at 37 °C.
4. Spin down cells, resuspend in fresh medium, and incubate for 2 days.
5. Spin down cells, count desired number into a fresh flask or wells of a plate, and infect as desired.