Time: 2024.05.10-2024.05.11

---

1. **Experiment:** Infection of NK92 cell line
2. **Time:** 2024.05.10-2024.05.11
3. **Member:** Hanyue Liu, Xudong Tang, Xiaoyuan Chen
4. **Material:** 5% FBS, complete medium (RPMI-1640 with 10% FBS), polybrene.
5. **Method:**

(1) Cell Inoculation:

① Collect NK92 cells from a culture flask.

② Centrifuge at 300×g for 5 minutes at room temperature.

③ Carefully remove the supernatant and resuspend the cell pellet in an appropriate volume of complete medium.

④ Calculate the required volume of cell suspension to achieve a final concentration of 1×10^7 cells/mL.

⑤ Add the calculated volume of cells to an appropriate volume of complete medium to achieve a final concentration of 1×10^7 cells/mL.

⑥ Dispense 100 μL of the 1×10^7 cells/mL suspension into each well of a 96-well plate. Incubate the plates at 37°C in a 5% CO_2 incubator for 24 hrs to achieve approximately 50% confluence using a light microscope to observe the cells. At 50% confluence, the cells should cover approximately half of the well surface. You'll see some areas with dense cell layers and others with less or no cells.

(2) Infection:

To each well, 4μL of a 25× polybrene infection-enhancing solution was added. Considering the multiplicity of infection (MOI) and the virus titer, the specific volume of the virus, calculated as (MOI × cell number) / virus titer, was added to the wells. The plates were then incubated at 37°C for 12-16 hrs.