Time: 2024.06.21-2024.06.22

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1. **Experiment:** Infection of CAR on NK92 cell line
2. **Time:** 2024.06.21-2024.06.22
3. **Member:** Hanyue Liu, Yaqi Gao
4. **Material:** Centrifuge 5810 R (Eppendorf, 5810R), Eclipse TS100 (Nikon, TS100), 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 mins at room temperature.
      ③ Carefully remove the supernatant and resuspend the cell pellet in an appropriate volume of complete medium (e.g., RPMI-1640 with 10% FBS).
      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, 4 μL 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.