

Time: 2024.06.23-2024.06.24

1. **Experiment:** Cell expansion and flow cytometry sorting
2. **Time:** 2024.06.23-2024.06.24
3. **Member:** Song Zhang, Xudong Tang, Hanyue Liu
4. **Material:** RPMI-1640 medium (10-15 mL per culture flask), FLAG-APC antibody (BioLegend, 637308) at 10 μ L per 2200 μ L of PBS (1:200 dilution) for initial labeling and 2 μ L per 1 mL of PBS (1:500 dilution) for secondary labeling, Phosphate-buffered saline (PBS).
5. **Method:**
 - (1) NK92 cell suspension from the stock is transferred to a tissue culture flask containing RPMI-1640 medium. An appropriate seeding density of $2-5 \times 10^6$ cells per 10-15 mL of medium should be ensured.
 - (2) The culture flask is placed in a CO₂ incubator set at 37°C with 5% CO₂ for 24 hrs. Cell growth should be monitored to ensure that cells are in the logarithmic phase.
 - (3) Replace the medium by removing the old medium and adding fresh RPMI-1640 medium.
 - (4) When cells reach 70-80% confluency, cells are passaged. The cell suspension is collected, and centrifuged at $300 \times g$ for 5 mins. The supernatant is discarded, and the cells are resuspended in a fresh medium before seeding into new culture flasks.
 - (5) Cells should be regularly counted and their morphology checked to ensure healthy growth.
 - (6) NK cells suspension was expanded to over 10^7 and centrifuged at 1500 rpm for 5 mins. 30 mL of PBS was used to wash the cells.
 - (7) The cells were resuspended by 2200 μ L of PBS and 10 μ L of FLAG-APC antibody (BioLegend, 637308) was added to label the cells. The cells were then incubated in the dark for 15 mins.
 - (8) The cells were washed again by 30 mL of PBS. The supernatant was discarded, and the cells were resuspended again by 500 μ L of PBS.
 - (9) The positive cells were sorted based on the CAR expression, from top 10% to 20%.
 - (10) The sorted cells were cultured to proliferate to 2×10^7 and the CAR-positive cell proportion is detected by FACS.
 - (11) 1 mL of cell suspension was taken. After the three-mins placement on ice, the cells were centrifuged at 3000 rpm for 5 mins and collected.
 - (12) The supernatant was discarded completely and the cells were resuspended in 1 mL of PBS.
 - (13) 2 μ L of FLAG-APC antibody (BioLegend, 637308) was added to label the cells.
 - (14) The cells were then incubated in the dark at room temperature for 15 mins. 1 mL of PBS was added to terminate the reaction and the cells could be collected after centrifuging at 3000 rpm for 5 mins.
 - (15) The cells were resuspended by 1 mL of PBS and the supernatant was discarded. The cells were resuspended in 50 μ L of ice-cold PBS, and flow cytometry analysis could be carried on.