SynthImmunol_NMU Notebook No.3

Time: 2024.07.15-2024.07.21

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1. **Experiment:** Molecules on the cells' surface detected by the flow cytometry
2. **Time:** 2024.07.15-2024.07.21
3. **Member:** Song Zhang, Xudong Tang, Hanyue Liu, Xiaoyuan Chen, Xinyu Zhu, Yinran Luo, Yaqi Gao
4. **Material:** FACS buffer, Fc receptor antibody (BioLegend), fluorescent antibody (BioLegen), PBS.
5. **Method:**
   (1) Preparation of Cell Suspension Samples: The effector cells were collected after 4 hrs of co-culture with tumor cells. Then, they were centrifuged 1×10^5 to 1×10^7 at 4°C for 5 mins at 250 g and the supernatant was discarded. The cells are resuspended in 100 μL of FACS buffer.
   (2) Preparation of Experimental Groups: Experimental groups, a blank control group, an isotype control group, and a single stain control group were set up.
   (3) The blocking of Fc Receptors on Cell Surface: 0.5 μL of Fc receptor antibody was added and the mixture was incubated in the dark at 4°C for 30 mins.
   (4) The binding of fluorescent antibodies to Cells: 1 μL of fluorescent antibody was added and the mixture was incubated in the dark at 4°C for 30 mins.
   (5) Pre-cooled PBS was added to remove unbound fluorescent antibodies. Then the cells were centrifuged at 1500 g for 5 mins and the supernatant was discarded. This step was repeated twice.
   (6) The cells were resuspended in 500 μL of PBS solution before loading.
   (7) Flow cytometry analysis is performed.