ADMEbio
for proteomic research

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Flow cytometry (FC/FCM/FACS) Protocol

I. Materials and Reagents:

- Cell and culture medium and hemocytometer
- 1X PBS (pH7.4)
- Fixing solution: 2% paraformaldehyde
- Penetration solution: precooling methanol
- Blocking buffer: 2% BSA /1xPBS
- Primary antibody and fluorochrome-conjugated second antibody

II. Experimental Procedure:

1. Cell collection:

- Collect the cultured cell.
- Count cells and adjust the cell concentration to 1~5×106/ml.

2. Wash:

- Add 8ml of PBS to resuspend the cells, vortexing gently.
- Centrifuge at 1000~2000rpm for 5 minutes.

3. Fix:

- Remove the supernatant.
- Add 6ml of 2% paraformaldehyde and mix thoroughly.
- Incubate at room temperature for 10 minutes or 4°C for overnight.

4. Wash:

- Centrifuge at 1000~2000rpm for 5 minutes.
- Discard the supernatant.
- Add 8ml of PBS and mix thoroughly.
- Centrifuge at 1000~2000rpm for 5 minutes.

5. Cell penetration (for intracellular staining):

- Remove the supernatant.
- Add 6ml of precooling methanol.
- Resuspend the cell with gentle vortexing.
- Incubate for 10 minutes at -20°C.

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6. Wash:
   - Centrifuge at 1000~2000rpm for 5 minutes.
   - Discard the supernatant.
   - Add 8ml of PBS and resuspend cells with gently vortexing.
   - Centrifuge at 1000 ~ 2000rpm for 5 minutes.

7. Block:
   - Remove the supernatant.
   - Add 1ml of 2% BSA and mix well.
   - Incubate at room temperature for 30 minutes.

8. Cell packing:
   - According the numbers of antibodies to detect, divide 1ml of the suspended cells into each tube.

9. Primary antibody:
   - Centrifuge at 1000~2000rpm for 5 minutes.
   - Discard the supernatant.
   - Add 0.1ml diluted primary antibody to each tube and mix well.
   - Incubate at 37°C for 60 minutes.

10. Wash:
    - Centrifuge at 1000~2000rpm for 5 minutes.
    - Discard the supernatant.
    - Add 1ml of PBS and mix well.
    - Centrifuge at 1000~2000rpm for 5 minutes.
    - Remove the supernatant.
    - Repeat one time starting from 10.3.

11. Secondary antibody*:
    - Add 0.1ml diluted secondary antibody to each tube and mix well.
    - Incubate at room temperature for 40 minutes.* avoid light using foil paper etc.

12. Wash:
    - Centrifuge at 1000~2000rpm for 5 minutes.
    - Discard the supernatant.
    - Add 1ml of PBS and mix well.
    - Centrifuge at 1000~2000rpm for 5 minutes.
    - Remove the supernatant.
    - Repeat once from 12.3.

13. Analysis:
    - Resuspend the cells in 0.2ml of PBS and mix well for flow cytometric analysis.