The preparation process of human peripheral blood PBMC

1) Collect anticoagulated human peripheral blood, dilute it with 1640 serum free medium or PBS at a ratio of 1:1, and mix upside down or by pipetting.

2) Add 3 mL of well-mixed Ficoll solution (1.077 g/mL) (Ficoll is taken out of the 4°C refrigerator half an hour in advance and returned to room temperature. If the temperature is too high, the separation will not be obvious, and if the temperature is too low, the density will be too large, and the separation effect will also be poor. The separation effect is best at about 20~25°C) to a 15 mL centrifuge tube, then add 2 mL of diluted blood along the tube wall slowly. The obviously stratification of blood and Ficoll liquid represents successful preparation.

3) Transfer the sample to a centrifuge and centrifuge at 500 g for 25 min.

4) Take out the centrifuge tube, and suck up mononuclear cells in the middle white film layer.

5) Wash the obtained mononuclear cells with 10 mL of PBS, centrifuge at 250 g for 10 min then discard the supernatant.

6) Repeat the wash step once.

7) Cells are resuspended for later use.

Human peripheral blood single cell suspension preparation process

1) Collect human peripheral blood samples in anticoagulant tubes.

2) Add 100 μL of fresh blood and 2 mL of 1× red blood cell lysate to the centrifuge tube, mix and lyse at 4°C for 10 min.

3) Centrifuge at 300 g for 5 min (centrifuge immediately after lysis to prevent cells damage), discard the supernatant, obtain white cell pellet.

4) Wash once with PBS.

5) Add 100 μL of PBS to resuspend the cells, and directly perform subsequent flow-cytometryantibody staining experiments without counting. Add 1 Test corresponding flow-cytometry antibody to single cell suspension prepared from 100 μL of fresh blood sample and mix well to conduct experiment.