Protocol of chemotaxis assay of THP1

A. Preparation of chemokine mother liquor

1. Short centrifuge chemokine dry powder

2. The mother liquor of 7 chemokines was prepared:

Volume of sterile water: 6.33 μl (CXCL1, cxcl2, cxcl3)

5.95 μl（CXCL8）

5.75 μl（CCL2）

6.41 μl（CCL3）

5.62 μl（CCL8）

The mother liquor of 1E5 nmol / L of seven chemokines was obtained

3. 1 μl mother liquor of each chemokine was added into the EP tube containing 999 μl medium to obtain 1 μl dilution

4. Dilution 1 of each chemokine: 200 μl was added into the EP tube containing 1800 μl medium to obtain dilution 2 of 10 nmol / L

B. Cell suspension was prepared

1. Select 5 THP1 culture dishes, take out the total volume of 25 ml cell suspension, take out 10 μl suspension for cell counting plate count, estimate cell density and suspension volume

2. 800 rpm, 5 min centrifugation, discard the supernatant, add 8.4 ml medium to resuspend cells

C. Transwell Migration Assay

1. 200 μl cell suspension was added into the upper chamber of each small hole, and 600 μl medium or chemokine solution was added into the lower chamber

Sampling order:

First board: first row: 1Control 1, 1Control 2, 1Control 3, cxcl1-1, cxcl1-2, cxcl1-3

The second row: 1Control 3, 1Control 4, 1Control 5, cxcl2-1, cxcl2-2, cxcl2-3

Starting time: 10:12

The second board: the first row: 2control 1, 2control 2, 2control 3, cxcl3-1, cxcl3-2, cxcl3-3

The second row: 2control 4, 2control 5, 2control 6, cxcl8-1, cxcl8-2, cxcl8-3

Starting time: 10:42

The third board: the first row: 3control 1, 3control 2, 3control 3, ccl2-1, ccl2-2, ccl2-3

The second row: ccl3-1, ccl3-2, ccl3-3, ccl8-1, ccl8-2, ccl8-3

Starting time: 10:24

3. Put it into the incubator for overnight cultivation