

In vitro Th0, Th1, Th2, Th17 and Th22 cell polarizations

@-CD3 coated (clone: 145.2C11) (1µg/ml) + @-CD28 coated (clone: 37.51) (1µg/ml) in PBS OVN 4°C in 50ul for 96 wells/plates.

Use 200ul in 96 wells/plates U bottom plates for a total of 50.000 pure CD4 T cells -->

Culture for 5 days!

Th0

Coated anti-CD3/CD28

Th1

Coated anti-CD3/CD28

+ IL-12 (5ng/ml)

+ anti-IL-4 (clone: 11B11) (10µg/ml)

Th2

Coated anti-CD3/CD28

+ IL-4 (10ng/ml)

+ anti-IFN-γ (clone: XMG1.2) (10µg/ml)

Th17

Coated anti-CD3/CD28

+ TGF-β (2ng/ml) + IL-6 (50ng/ml) + IL-1β (20ng/ml) + IL-21 (100ng/ml) + IL-23 (20ng/ml)

+ anti-IL-4 (clone: 11B11) (10µg/ml) + anti-IFN-γ (clone: XMG1.2) (10µg/ml)

Th22

Coated anti-CD3/CD28

IL-6 (50ng/ml) + IL-23 (50ng/ml)

+ anti-IL-4 (clone: 11B11) (10µg/ml) + anti-IFN-γ (clone: XMG1.2) (10µg/ml)

Day 3 look at the cells and add some fresh medium if really yellow.

Day 5 PMA-Iono restim and intracellular FACS staining.