PKC and erk (MAPK) staining

TALLs: 0.25~0.5million/condition in 250ul medium

Conditions: stim, unstim, no primary antibody, compound treated samples

Procedure:

1. incubate cells with compound at certain final concentrations, 30min, RT
2. + stim/unstim solution to correspondent samples, 50min, RT, on rotor (unstim solution for unstim condition, stim solution for everything else)
3. +100ul 4%PFA, 20min, RT
4. +1ml methanol (ice cold, in -20C freezer), 20min, 4C
5. spin 13200rpm, 5min; aspirate; +1ml FACS buffer wash; spin 13200rpm, 5min, aspirate
6. +primary Ab, 1:1000 dilution in 1ml FACS buffer, 100ul each (except for no primary one), 20min, RT
7. +1ml FACS buffer wash; spin 13200rpm, 5min, aspirate
8. +secondary Ab, 1:100 dilution in FACS buffer, 100ul each, 20min, RT
9. +1ml FACS buffer wash; spin 13200rpm, 5min, aspirate
10. +400ul 1% PFA, flow cytometer analysis

Solutions:

stim (10ul each)

TG 1ul

PMA 1ul

medium 38ul

unstim (10ul each)

medium 40ul

Ab\*:

|  |  |  |
| --- | --- | --- |
| target | erk(MAPK) | PKC |
| primary Ab | mouse α human active MAPK mAb (in -20C, "cell signaling"box) | rabbit α human active PKC substrate Ab (in -20C, "cell signaling"box) |
| secondary Ab | Cy5 conjugated donkey α mouse (in -20C, "2 Ab A-M"bucket) | FITC conjugated donkey α rabbit (in -20C, "2 Ab N-Z"bucket) |
| flow channel | FL-4 | FL-1 |

\*diff. targeted Ab can be mixed together during incubation, but not primary & secondary mixed together.