Flow cytometry sample preparation

(22. 7. 14)

현종 & 지수

- 세포 수를 약 50,000개(보통의 경우) 검출할 예정이기 때문에 세포 수를 counting하는 것이 좋다.
- 12 well 당 trypsin 100ul
- FACS buffer에서 sodium azide의 역할: ①buffer의 방부제, ②큰포식세포의 식작용 억제(항체)
- Single staining sample도 반드시 준비해야함

1. Cell preparation

1) 12 well trypsin 100ul 사용하여 세포를 모운다(1300rpm 3 min) (including neutralization step).
2) Add 200ul of FACS buffer for washing (V bottom) and resuspension.
3) Centrifugation (2000rpm, 3min)
4) Discard supernatant.

2. FcγR blocking

1) Add 50ul (per well, V bottom) of FcγR blocking solution (1 : 500 = FcγR antibody : FACS buffer) and resuspension.
2) Incubation in ice for 30 min
3) Add 200ul of FACS buffer (total: 250ul) and centrifugation (2000rpm, 3min).
4) Discard supernatant.

3. Flow cytometry staining

*Fixation (with fixation buffer)

1) **Fix buffer** 200ul 분주 후 suspension
2) Incubation in ice for 20 min
3) 20분 이후 2000rpm 3min
4) Discard supernatant

*Surface marker staining (dark condition)

5) Add 50ul of antibody cocktail (per well, V bottom) (commonly 200-500-fold dilution in FACS buffer) and resuspension.
6) Incubation in ice for 30 min.
7) Add 200ul of FACS buffer (total: 250ul) and centrifugation (2000rpm, 3min).
8) Discard supernatant.

*Permeabilization (with permeabilization buffer)

9) Add 200ul of **Perm buffer** and suspension.
10) Incubation in ice for 30min
11) 30분 이후 2000rpm 3min centrifuge

12) Discard supernatant

*Intracellular marker staining (dark condition)

13) Add 50ul of antibody cocktail (per well, V bottom) (commonly 200-500-fold dilution in **Perm buffer**) and resuspension.

14) Incubation in ice for 30 min.

15) Add 200ul of **Perm buffer** (total: 250ul) and centrifugation (2000rpm, 3min).

16) Discard supernatant.

17) Add 200ul of FACS buffer and resuspension.

18) FACS tube에 옮기기

4. Flow cytometry operating

1) [Cytometry setting] → Fluorescence selection

2) [Compensation settings] → create compensation controls