Intracellular FACS staining using Paraformaldehyde and Saponin

1. Stimulation with PMA (final at 50ng/ml) and Ionomycine (final at 1ug/ml) for 4h at 37oC. After 2h add Brefeldin A (final at 10ug/ml).

2. Transfer into V bottom well plates.

3. Wash once in FACS buffer + Brefeldin A.

4. Resuspend in 100ul FACS buffer + Brefeldin A and add 100ul of Paraformaldehyde 4%. PAF4% tubes contain 2ml, calculate how many tubes needed.

Incubate 15min at 4oC.

5. Wash twice in FACS buffer + Brefeldin A and centrifuge 5min at 2000rpm from now on.

6. Wash once in FACS buffer + Saponin 0.5% (final).

7. Incubate 20 min at RT with FACS buffer + Saponin 0.5% (final) + Fc Block + 5% NMS.

8. Without washing add intracellular Ab (1/100) in FACS buffer + Saponin 0.5% (final).

Incubate 30 min at RT.

9. Wash once in FACS buffer + Saponin 0.5% (final).

10. Wash once in FACS buffer.

11. Cells are ready to go to the FACS.