

## Recommended Staining Protocol for CyTOF (PBMC)

1. Thaw PBMC, washing once with medium (RPMI/10%FCS) and filter
2. Suspend in medium, and stimulate with PMA/ionomycin for 15min
3. Stop the reaction by adding 1/10 volume of 16% PFA
4. Dispense 3 million cells/ FACS tube after washing twice with FACS buffer
5. Surface stain with Fluidigm PBMC panel kit for 30 min at RT, dark (in 100ul, 1/50 dilution)
6. Wash once with FACS buffer and aspirate pellet to < 100ul
7. Suspend the cells in 1 ml 100% Methanol (4C) and incubate on ice for 10 min
8. Wash 3 times with permeabilization buffer from eBioscience's Foxp3 staining kit
9. Intracellular staining at RT for 30 min in permeabilization buffer (in 100ul, 1/50 dilution), then wash once
10. 1.6% PFA/PBS containing Iridium 1:4000 overnight (4°C, dark) (in 1ml)