Ritchlin Laboratory

Antibody staining of PBMC and Monocyte for Flow Cytometry and Cell culture differentiation of OCPs.

Date: 12JANUARY2018
1.0 Subject
Intracellular and extracellular antibody staining.

2.0 Purpose
Staining of cells isolated from whole blood using extracellular and intracellular antibodies in testing the presence and expression of membrane bound proteins using Flow Cytometry.

3.0 Scope
The scope of this procedure applies to the R01 Grant provided by the NIH for BSL-2 operations in the Ritchlin laboratory.

4.0 Safety Concerns
Working with patient blood and sera are considered BSL-2.

5.0 Definitions

6.0 Materials

6.1 Antibodies (List attached at end with cat #’s)
1. CXCR5 Rat Anti-Human
2. CCR6 (CD196) Mouse Anti-Human
3. CD14 Mouse Anti-Human
4. Live/Dead CD16 Mouse Anti-Human
5. CD278 Mouse Anti-Human
6. CD279 Mouse Anti-Human
7. CD3 Mouse Anti-Human
8. CD19 mouse Anti-Human
9. CD16 Mouse Anti-Human
10. CD8 Mouse Anti-Human
11. CD4 Mouse Anti-Human
12. DC-STAMP APC Mouse Anti-Human
6.2 Consumables
1. 5mL polystyrene flow tube
2. 1X PBS w/ 0.5-1%BSA or 5-10% FBS
3. FcX Human Blocking Reagent
4. OCP media: CM media (a-mem, 10%FBS, 1% pen/strep) with 10ng/ul hM-CSF (1000X) and 10ng/ul hRANKL (1000X).
5. Live/Dead aqua stain (Thermo Fisher Cat# L34965).
6. Flow Cytometry Staining Buffer (R&D FC001)

7.0 Procedure
7.1 FOR Cell culture of OCP:
   7.1.1 Resuspend cells (P1, P2, M1, M2) in 350ul of OCP Media.
   7.1.2 Pre-label 96 well plate. Add total volume of cells between two wells for each.
   7.1.3 Store in 37’ incubator for 4 days, then replace media. Stain on the 7th day.

7.2 For Flow Cytometry staining:
   7.2.1 For UNSTAINED tube ONLY, add 100ul of Fix solution. 15min, RT.
   7.2.2 For All other tubes, resuspend cells in 50 ul of PBS containing 1ul Live/Dead aqua stain (blue top), 25 min RT.
      7.2.2.1 New live/Dead vial. Resuspend in 50ul DMSO (green top)
   7.2.3 Wash, 1400rpm, 5min.
   7.2.4 Resuspend in Human Trustain FcX Fc blocker (Biolegend cat# 422302) 10min RT.
   7.2.5 Create a master mix of all antibodies (5ul each per ab, 10ul for CD8) excluding DCSTAMP and live/dead.
   7.2.6 Add respective amount ab cocktail to cells, 20 min RT protected from light.
   7.2.7 Wash w/ PBS.
   7.2.8 Fix cells with 100ul fix buffer (Thermo cat# GAS001S), 30 min RT.
   7.2.9 Wash w/ PBS.
7.3 Intracellular Staining
   7.3.1 Resuspend cells in 50ul Permeabilization (Thermo Fisher cat# GAS002S5) along with 8ul of DCSTAMP ab. 20min RT, protected from light.
   7.3.2 Wash w/ PBS.
   7.3.3 Resuspend cells in 350ul Flow Cytometry staining buffer.
   7.3.4 Store at 4’.