

**Ritchlin Lab**  
**Standard Operating Procedure**

Number: A1  
Revision: A  
Effective Date: 12January2018

Title: Antibody staining of PBMC and  
Monocyte for Flow Cytometry

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Ritchlin Laboratory

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

Date: 12JANUARY2018

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### 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'.

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