Flow Microwell Staining—Dendritic Cell Panels

Supplies:

1. 200 mcL round-bottom plate
2. PDBS + 4% FCS (washing solution)
3. PFA 1% (poison)
4. antibodies
5. microcentrifuge tubes to mix antibodies
6. flow tubes with lids

Panels

Table 1: Dendritic Cell Panel #1: Used March 14/18/19. Was panel #2 on 3/14.

FITC	FL1-H	BDCA-2 / CD303
PE	FL2-H	CD14
PECy5	FL3.H	HLA-DR
APC	FL5 (FL2-W)	BDCA-4 / CD304

Table 2: Dendritic Panel #2. March 20, 2008 onward.

FITC	FL1-H	BDCA-2 / CD303
PE	FL2-H	CD14
PerCP	FL3.H	CD45

Table 3: Dendritic Panel #3. March 20, 2008 onward.

FITC	FL1-H	BDCA-2 / CD303
PE	FL2-H	BDCA-1 / CD1c
PerCP	FL3.H	CD45

Table 4: Dendritic Panel #4. March 20, 2008 onward.

FITC	FL1-H	HLA-DR
PE	FL2-H	CD14
PerCP	FL3.H	CD45

Table 5: Dendritic Panel #5. March 20, 2008 onward.

FITC	FL1-H	HLA-DR
PE	FL2-H	CD14
PerCP	FL3.H	CD45
APC	FL5 (FL2-W)	BDCA-3 / CD141

Table 6: Dendritic Panel #6. March 20, 2008 onward.

FITC	FL1-H	HLA-DR
PE	FL2-H	BDCA-1 / CD1c
PECy5	FL3.H	CD45
APC	FL5 (FL2-W)	BDCA-3 / CD141

Surface Staining: *(< 60 minutes)*

1. Get sample into each well (each well will become a flow tube)

1.1. place 200 mcL of sample into each well (dilute with washing solution)

1.2. spin plate 1200rpm x 2 minutes

1.3. flip drip dry

1.4. wipe off top

1.5. agitate via quick vortex

2. Stain the cells

2.1. add 16 mcL of washing solution

2.2. add 2.5 mcL of each antibody to wells

2.3. mix with 10 mcL pipet (up and down 5—10 times)

2.4. incubate at 4 °C for 30 minutes

3. Wash antibody off of cells

3.1. add 200 mcL of washing solution

3.2. spin plate 1200rpm x 2 minutes

3.3. flip drip dry

3.4. wipe off top

3.5. agitate via quick vortex

4. Fix cells

4.1. add 200 mcL 1% PFA to each well

4.2. fill each flow tube with 300 mcL PFA

4.3. add contents of wells to tube

4.4. vortex tubes

4.5. cover with cap, keep at 4 °C until ready to use