

## Site Density Protocol

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**[Abstract]** The site densities of cell surface molecules provide useful information for cell function analysis. Using antibody staining and commercially available calibration beads, this assay quantitatively determines the T cell receptor site density at the single T cell level. This method can be easily extended to quantify other surface molecule densities on different cells or beads.

### Materials and Reagents

1. OTI T cells
2. PE-conjugated anti-mouse TCR Va2 monoclonal antibody B20.1 (BD Biosciences)
3. PE Rat IgG2a,  $\lambda$  Isotype Control (BD Biosciences)
4. EDTA
5. BSA
6. PBS
7. Sodium azide
8. FACS staining buffer (see Recipes)

### Equipment

1. Countertop centrifuges
2. BD LSR flow cytometer
3. Shaker
4. QuantiBRITE PE tube (BD Biosciences)

### Procedure

1. OTI T cells ( $1 \times 10^5$ ~ $1 \times 10^6$ ) were incubated with anti-TCR Va2 antibody or isotype control antibody at 10  $\mu\text{g/ml}$  (or saturated concentration) in 200  $\mu\text{l}$  of FACS buffer at 4  $^\circ\text{C}$  for 30 min on a shaker.
2. Wash three times with cold FACS buffer by centrifuge at 500 x g for 3 min.
3. Resuspend the T cells in 400  $\mu\text{l}$  cold FACS buffer.
4. Add 400  $\mu\text{l}$  cold FACS buffer into QuantiBRITE PE tube, and gently shake the tube to resuspend the beads.

5. Measure the fluorescence intensities of T cells and QuantiBRITE PE beads by a BD LSR flow cytometer.
6. Plot a linear regression of PE molecules per bead against measured mean fluorescence, using the following equation:

$$y = mx + c$$

Where y equals measured mean fluorescence and x equals PE molecules per bead provided by manufacturer; m is slope and c is the intercept.

7. Use above equation to calculate the total number of molecules per cell according to measured T cell mean fluorescence (after subtract isotype control fluorescence) and the antibody F/P (the number of fluorochrome molecules per Ig molecule) molar ratio, and divided by the T cell surface area to obtain the site density.

### Recipes

1. FACS staining buffer  
PBS  
5 mM EDTA  
1% BSA  
0.02% sodium azide

### References

1. Huang, J., Zarnitsyna, V. I., Liu, B., Edwards, L. J., Jiang, N., Evavold, B. D. and Zhu, C. (2010). [The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness.](#) *Nature* 464(7290): 932-936.