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, λ 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 (1×10^5~1×10^6) were incubated with anti-TCR Va2 antibody or isotype control antibody at 10 μg/ml (or saturated concentration) in 200 μl of FACS buffer at 4 °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 μl cold FACS buffer.
4. Add 400 μl 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.

