Cell Surface Staining Protocol with MultiDots

Introduction

MultiDots are polymer-protected nanoparticles that encompass inorganic semiconductor nanocrystals. They have increased sensitivity, high fluorescence intensity, resistance to photobleaching, and improved shelf-life stability. A narrow emission spectrum and distinct spectral signature of the MultiDots eliminates the need for complex spectral compensation. For a high-dimensional assay, this allows for the accommodation of more fluorophores within a panel with reduced spread error. MultiDots ease end-users' workflow, reduces duration of procedure, and improves staining quality.

Reagents and Instruments

- Target cell suspension in PBS or buffer of choice
- MultiDot antibody
- Staining Buffer: PBS + 0.5% BSA+ 2 mM EDTA
- 12x75 mm round bottom polystyrene tubes, 50 mL centrifuge tube
- Centrifuge, Vortex, Flow Cytometer

Direct Labeling

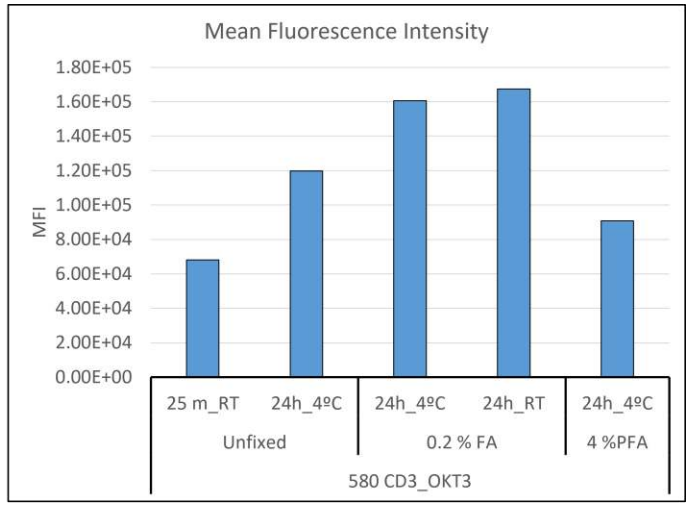
Sample Preparation: Prepare single-cell suspension in staining buffer with desired concentration of cells.

1. Resuspend 1E+06 cells in 200 µL of staining buffer in a flow tube.
2. Add **10 µL of MultiDot antibody conjugate for a million cells**
3. Pipette briefly to mix the conjugate with the cells.
4. Incubate for 25 minutes at room temperature.
   Note: Refer the optimal labeling conditions for improved MFI, and staining index(SI), Fig 1.
5. Wash cells by adding 1 mL of staining buffer to the flow tube, followed by centrifugation at 1800 rpm for 5 minutes.
6. Remove the supernatant completely.
7. Resuspend the pellet in 350 µL of staining buffer and analyze in flow cytometer.

Optimal labeling conditions for improved MFI and Staining Index (SI)

PBMCs from fresh blood were labeled with MultiDot antibody conjugates and the effect of staining time (*25 minutes vs 24 hrs)*, temperature (*4ºC vs room temperature (RT)*) and fixatives (*0.2% formalin and 4 % paraformaldehyde (PFA)*) were studied.

PBMCs **fixed with 0.2% formalin and stained overnight with 10 uL MultiDots conjugate**, yielded improved staining index and MFI.



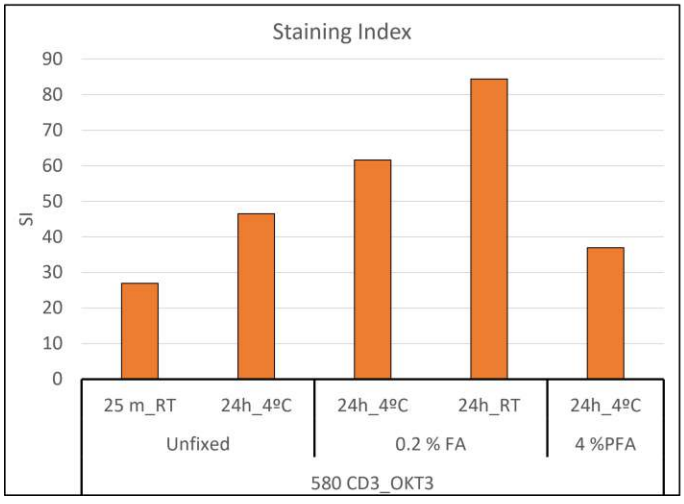


Figure 1. MFIs and SI of MultiDot 580 CD3 (OKT3) labeled PBMCs under various conditions analyzed in Cytek Aurora

855-278-2266
info@corequantum.com
www.corequantum.com