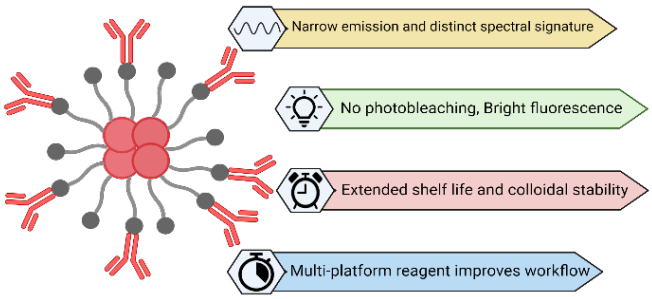
Cell Surface Staining Protocol with MultiDot Streptavidin or Anti-Biotin

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, reduce the procedure's duration, and improve the staining quality.



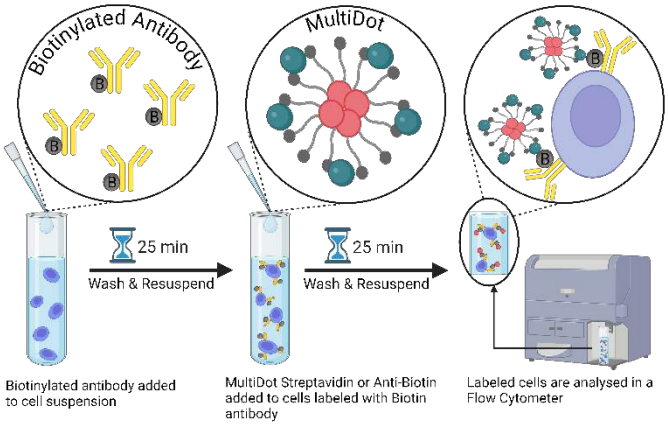
Reagents and Instruments

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

Two Step 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 and briefly mix appropriately titrated biotinylated antibody to cells in the flow tube.
3. Incubate for 25 minutes at room temperature.
4. Wash cells by adding 1 mL of staining buffer to the flow tube, followed by centrifugation at 1800 rpm for 5 minutes.
5. Remove the supernatant completely.
6. Resuspend the pellet with 200 µL of staining buffer.
7. Add appropriately titrated MultiDot conjugate (Streptavidin or Anti-Biotin) to the flow tube and pipette briefly to mix.
   Note: 10 µL of MultiDot conjugate for a million cells is recommended
8. Incubate for 25 minutes at room temperature.
   Note: If cells are very phagocytic, incubate at 4°C
9. Wash cells by adding 1 mL of staining buffer to the flow tube, followed by centrifugation at 1800 rpm for 5 minutes.
10. Remove the supernatant completely.
11. Resuspend the pellet in 350 µL of staining buffer and analyze in flow cytometer.



Other Applications

- Flow cytometry
- Fluorescent microscopy
- ELISA
- Immunohistochemistry