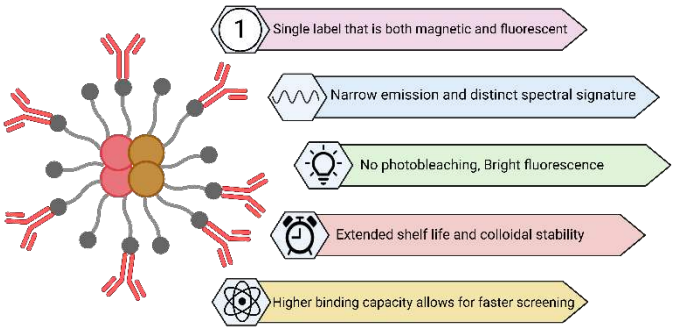
MagDot Streptavidin or Anti-biotin Labeling and Separation Protocol in Column-based Magnet

Introduction

MagDot is a unique reagent that combines inorganic semiconductor nanocrystals and superparamagnetic iron oxide particles within the same nanoparticle. They have increased sensitivity, high fluorescence intensity, resistance to photobleaching, and improved shelf-life stability. Owing to its dual nature, it is possible to perform cell separation on MagDot labeled cells via Magnet Activated Cell Sorting (MACS) and immediately transfer the cells to Fluorescence Activated Cell Sorting (FACS) for further purification without any additional fluorescent labeling procedure.



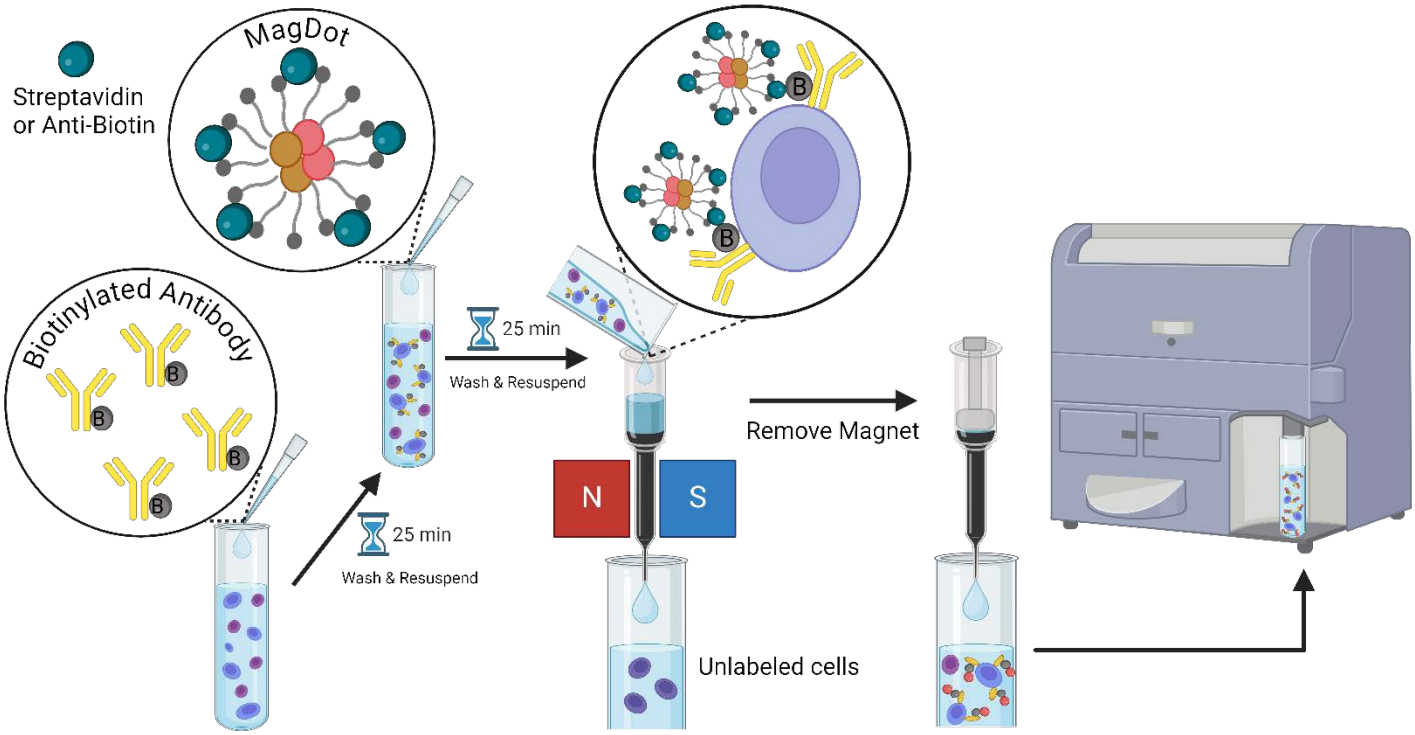
Reagents and Instruments

- Target cell suspension in PBS or buffer of choice
- Staining Buffer: PBS + 0.5% BSA + 2 mM EDTA
- Biotinylated antibody
- MagDot Streptavidin or MagDot Anti-Biotin
- 12x75mm round bottom polystyrene tubes, 50 mL centrifuge tube
- Centrifuge, Vortex, Flow cytometer
- Closed column and magnet

Two Step Labeling and Column-Based Separation

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 appropriately titrated biotinylated antibody to the flow tube and pipette briefly to mix.
3. Incubate for 25 minutes at room temperature.
4. Wash cells by adding 1 mL of the 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 MagDot conjugate (Streptavidin or Anti-Biotin) to the flow tube and pipette briefly to mix.
   Note: 25 µL of MagDot 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. Post incubation, add 300 µL of staining buffer to make the volume up to 500 µL.
10. Separation with column-based magnet
    a. Prime the column by adding 500 µL of staining buffer.
    b. Attach the column to the magnet.
    c. Place a tube under the column (this is for the unlabeled/non-magnetic cell population).
    d. Add the cell suspension mixture to the column and let it pass through.
    e. Add 1 mL of staining buffer to the column and allow it to pass through into the tube.
    f. Once all the buffer has passed, remove the column from the magnet.
    g. For the labeled (magnetic) cells, add 500 µL of staining buffer to the column.
    h. Using the plunger to purge the labeled cell population into another tube.
11. Directly analyze the cells in flow cytometer.



Biotinylated antibody added to cell suspension followed by MagDot Streptavidin or Anti-Biotin addition

Cell suspension mixture transferred into the column.

Labeled cells washed out from column and directly analysed in Flow Cytometer

*Image components are not to scale

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