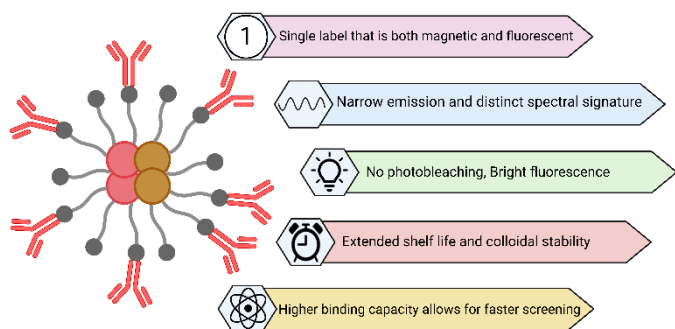


# MagDot Labeling and Separation Protocol in a Column-based Magnet

## 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
- MagDot antibody
- 12x75 mm round bottom polystyrene tubes, 50 mL centrifuge tube
- Centrifuge, Vortex, Flow cytometer
- Closed column and magnet

## Direct Labeling and Column-based Separation

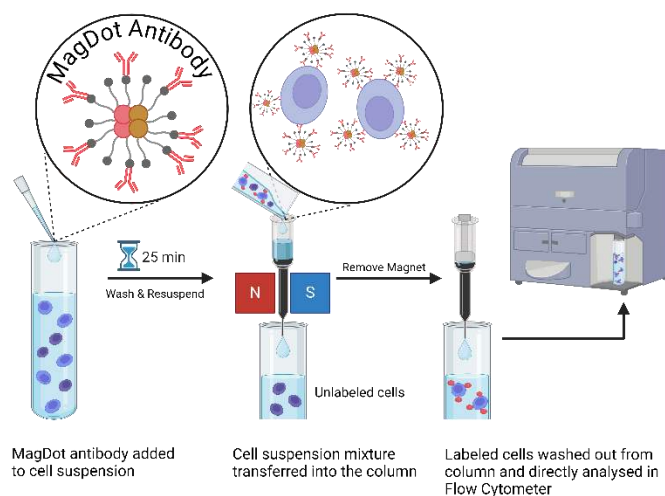
Sample Preparation: Prepare single-cell suspension in staining buffer with desired concentration of cells.

1. Resuspend  $1E+06$  cells in 200  $\mu$ L of staining buffer in a flow tube.

2. Add appropriately titrated MagDot antibody to the flow tube, and pipette briefly to mix.

*Note: 25  $\mu$ L of MagDot antibody for a million cells is recommended*

3. Incubate for 25 minutes at room temperature.  
*Note: If cells are very phagocytic, incubate at 4°C*
4. Post incubation, add 300  $\mu$ L of staining buffer to make the volume up to 500  $\mu$ L.
5. Separation with column-based magnet
  - a. Prime the column by adding 500  $\mu$ L 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  $\mu$ L of staining buffer to the column.
  - h. Use the plunger to purge the labeled cell population into another tube.
6. Directly analyze the cells in flow cytometer.



\*Image components are not to scale

