MojoSort™ Mouse anti-PE Nanobeads Column Protocol

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Abstract

BioLegend MojoSort™ nanobeads work in commonly used separation columns, based on our internal research as well as validation by external testing by academic labs. This simple protocol consists of following the MojoSort™ protocol to label the cells with **pre-diluted** MojoSort™ reagents and using the columns as indicated by the manufacturer.

Note: Due to the properties of our beads, it may be possible to use far fewer beads that with other commercial suppliers. We recommend a titration to find the best dilution factor. However, as a general rule, dilutions ranging from 1:3 to 1:20 for the Nanobeads can be used. Please contact BioLegend Technical Service (tech@biolegend.com) if further assistance is needed.

Guidelines

MojoSort™ magnetic particles can be used with other commercially available magnetic separators, both free standing magnets and column-based systems. Because MojoSort™ protocols are optimized for the MojoSort™ separator, the protocols may need to be adjusted for other systems. Please contact BioLegend Technical Service (tech@biolegend.com) for more information and guidance. We do not recommend using MojoSort™ particles for BD's IMag™ or Life Technologies' DynaMag™.

Materials

MATERIALS

MojoSort™ Buffer **BioLegend Catalog #**480017

MojoSort™ Mouse anti-PE Nanobeads **Catalog #**480079

PE-Conjugated Anti-Mouse Antibody

Additional reagents:
-commercially available cell separation columns
-5 mL polypropylene tubes
-70 µm cell strainer

1 Prepare cells from your tissue of interest or blood without lysing erythrocytes.

2 In the final wash of your sample preparation, resuspend the cells in MojoSort™ Buffer by adding up to 4 mL in a 5 mL (12 × 75 mm) polypropylene tube.
Note: Keep MojoSort™ Buffer on ice throughout the procedure.

3 Filter the cells with a 70 µm cell strainer, centrifuge at 300xg for 5 minutes, and resuspend in an appropriate volume of MojoSort™ Buffer. Count and adjust the cell concentration to 1 × 10^8 cells/mL by adding MojoSort™ Buffer. 5m

4 Aliquot 100 µL (10^7 cells) into a new tube. Check the recommended usage for flow cytometric staining of the PE-conjugated antibody indicated in the antibody technical datasheet. Calculate the volume to stain 10^7 cells (or desired amount of cells). **Add the appropriate volume of pre-diluted PE-conjugated antibody** to the cell suspension, mix well and **incubate on ice for 15 minutes.** 15m
Note: For the PE-conjugated antibodies, we recommend to do a titration to determine the optimal concentration.

5 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes. 5m

6 Discard the supernatant and resuspend cells in 100 µL of MojoSort™ Buffer.

7 Vortex the anti-PE Nanobeads (to resuspend) at max speed, 5 touches, and prepare the dilutions to test. **Add 10 µL of pre-diluted anti-PE Nanobeads**. Mix well **and incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells. For example, add 100 µL of pre-diluted Nanobeads for separating 1 × 10^8 cells in 1 ml of MojoSort™ Buffer. When working with less than 10^7 cells, use indicated volumes for 10^7 cells. 15m
Note: The amount of Nanobeads to use always depends on the frequency of the target, among a few other factors. We recommend to do a titration to determine the optimal concentration.

8 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes. 5m

9 Discard the supernatant.

10 Add the appropriate amount of MojoSort™ Buffer and proceed to separation. At least 500 µL is needed for column separation.

Note: There are several types of commercially available columns, depending on your application. Choose the one that fits best your experiment:

	Max. number of labeled cells	Max. number of total cells	Cell suspension volume	Column rinse volume	Cell wash volume	Elution volume
Small Capacity	1×10^7	2×10^8	500µL for up to 10^8 cells	1ml	1 ml	1 ml
Medium Capacity	1×10^8	2×10^9	500µL for up to 10^9 cells	3ml	3 ml	5 ml
Large Capacity	1×10^9	2×10^{10}	500µL for up to 10^{10} cells	20-50ml	30 ml	20 ml

Example of magnetic separation with medium capacity columns:

11 Place the column in a magnetic separator that fits the column.

12 Rinse the column with 3 mL of cell separation buffer.

13 Add the labeled cell suspension to the column through a 30 µm filter and collect the fraction containing the unlabeled cells.

14 Wash the cells in the column **3 times** with 3 mL of buffer and collect the fraction containing the unlabeled cells. Combine with the collected fraction from step 3. These cells may be useful as controls, to monitor purity/yield, or other purposes.

15 Take away the column from the magnet and place it on a tube. Then add 5 mL of buffer and flush out the magnetically labeled fraction with a plunger or supplied device. These are the positively isolated cells of interest; do not discard. To increase the purity of the magnetically labeled fraction repeat the isolation process with a new, freshly prepared column.