



MojoSort™ Mouse Ig light chain κ 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 in 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.

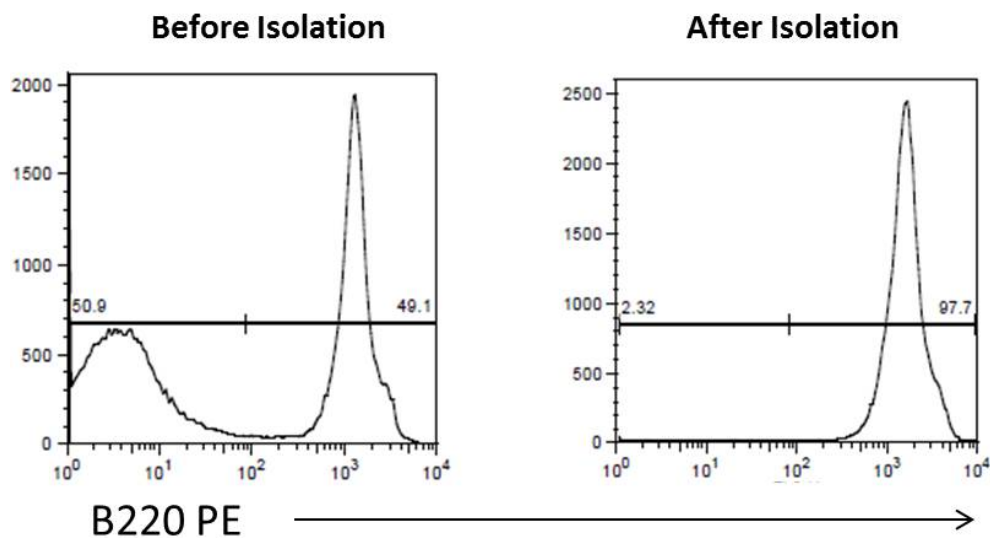
Note: Due to the properties of our beads, it may be possible to use far fewer beads than with other commercial suppliers. We recommend a titration to find the best dilution factor. However, as a general rule, dilutions ranging from 1:3 to 1:20 for the Nanobeads can be used. Please contact BioLegend Technical 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™.

Representative Data

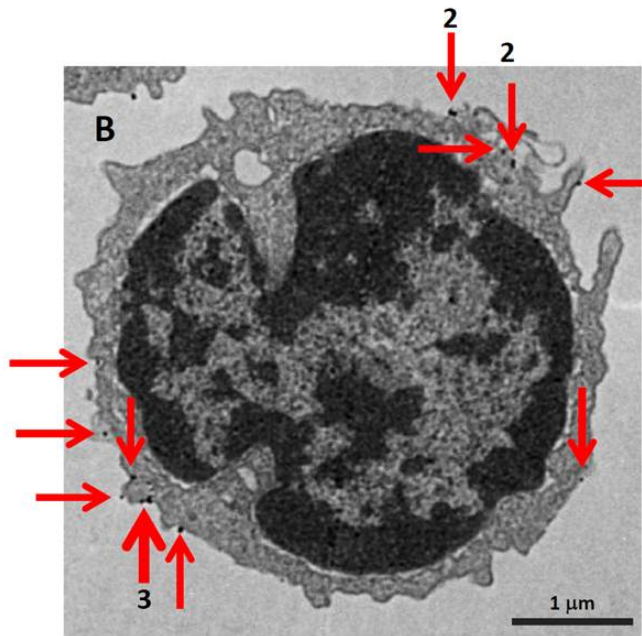
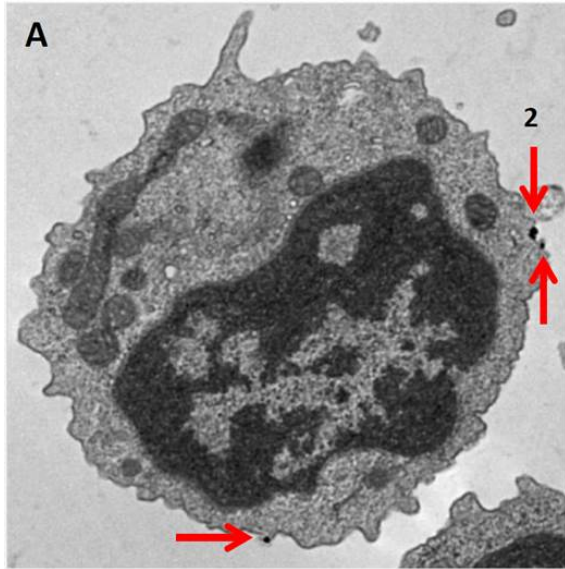
Flow cytometry. High purity and yield. "After Isolation" plot shows purified population of interest using pre-diluted MojoSort™ reagents in separation columns.



Kit	Purity	Yield
Mouse CD19 Nanobeads	97.7%	94%

Electron Microscopy. MojoSort™ Nanobead-isolated CD19+ cells using columns do not display more bound beads on the cell surface (A) as compared to cells isolated with a compatible commercial product using the same columns (B). Red arrows indicate where the particles are located. Numbers indicate either 2 or 3 magnetic

particles adjacent to each other. Pictures were taken at the same magnification, scale shown in B. Images are representative of 41 different cells each.



Materials

MATERIALS

MojoSort™ Mouse Ig light chain κ Nanobeads BioLegend Catalog #480125



- 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 70µm 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.
- 4 Aliquot 100µL of cell suspension (10^7 cells) into a new tube.
- 5 (*Optional*) Add 5µL of Human TruStain FcX™ (Fc Receptor Blocking Solution), mix well and incubate at room temperature for 10 minutes. Scale up the volume accordingly if separating more cells. For example, if the volume of Human TruStain FcX™ for 1×10^7 cells is 5µL, add 50µL for 1×10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.
- 6 Stain 10^7 cells using the mouse Ig light chain κ -containing antibody following manufacturer's instructions for flow cytometric surface staining. Scale up the volume accordingly if staining more than 10^7 cells. Note: For the mouse Ig light chain κ -containing antibodies, we recommend to do a titration to determine the optimal concentration.
- 7 Wash the cells by adding MojoSort™ Buffer up to 4mL. Centrifuge the cells at 300xg for 5 minutes.
- 8 Discard the supernatant and resuspend cells in 100uL of MojoSort™ Buffer.
- 9 Resuspend the beads by vortexing, maximum speed, 5 touches. Add the appropriate volume of pre-diluted Mouse Ig light chain κ Nanobeads to the cell suspension, mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells. For example, if the volume of Nanobeads for 1×10^7 cells is 10µL, add 100µL for 1×10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells. Note: The amount of Nanobeads to use always depends on the frequency of the target, among a few other factors. We recommend doing a titration to determine the optimal concentration.
- 10 Wash the cells by adding MojoSort™ Buffer up to 4mL. Centrifuge the cells at 300xg for 5 minutes.



- 11 Discard the supernatant.
- 12 Add the appropriate amount of MojoSort™ Buffer and proceed to separation. At least 500µL 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:

- 13 Place the column in a magnetic separator that fits the column.
- 14 Rinse the column with 3mL of cell separation buffer.
- 15 Add the labeled cell suspension to the column through a 30µm filter and collect the fraction containing the unlabeled cells.
- 16 Wash the cells in the column 3 times with 3mL 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.
- 17 Take away the column from the magnet and place it on a tube. Then add 5mL 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.