MojoSort™ Mouse CD8a Selection Kit Column Protocol

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Sam Li[1]

[1]BioLegend

BioLegend
Tech. support email: **tech@biolegend.com**

Sam Li
BioLegend

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Abstract

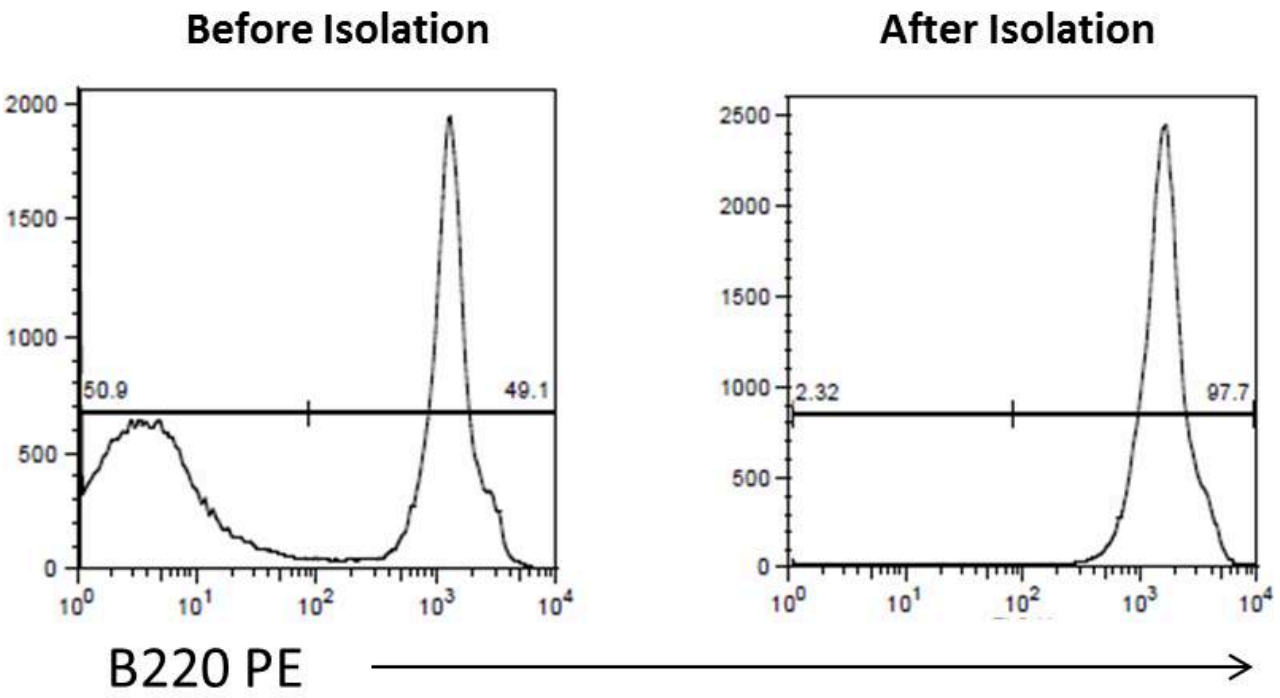
BioLegend MojoSort™ nanobeads work in commonly used separation columns, based on our internal research as well as validation by external testing by academic labs. This simple protocol consists of following the MojoSort™ protocol to label the cells with **pre-diluted** MojoSort™ reagents and using the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

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

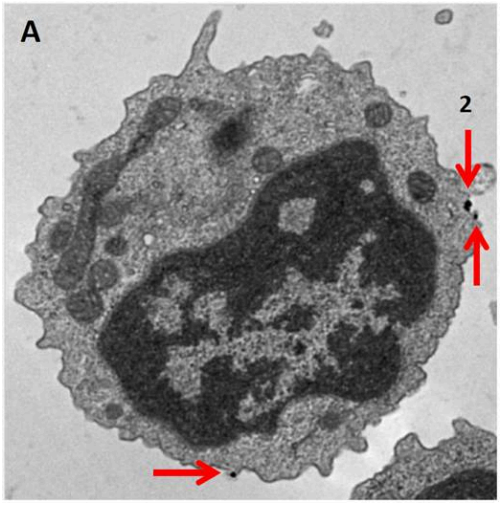
Flow cytometry. High purity and yield. "After Isolation" plot shows purified population of interest using pre-diluted MojoSort™ reagents in separation columns.



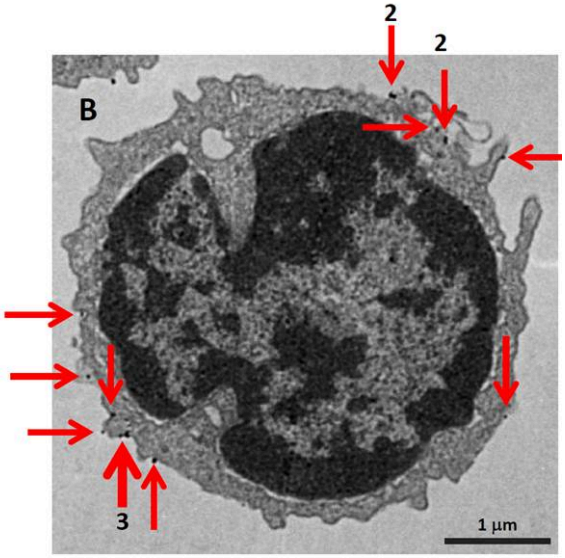
	Kit	Purity	Yield
	Mouse CD19 Nanobeads	97.7 %	94%

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.



B cells isolated with MojoSort™ CD19 nanobeads using separation columns.



B cells isolated with competitor's CD19 magnetic beads using separation columns.

Materials

MATERIALS

MojoSort™ Mouse CD8a Selection Kit **BioLegend Catalog #**480135

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 4mL 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 (10^7 cells) into a new tube. Prepare biotin antibody dilution by adding 5 µL of the Biotin anti-mouse CD8a antibody (100X) to 995 µL of 1X MojoSort Buffer (clarification: this is more than the 100X dilution indicated on the label, which is the suggested dilution for its use on the hand-held MojoSort™ magnet, not on columns).

5 Add 10µL of the pre-diluted Biotin-Antibody Cocktail to the sample. Mix well and incubate on ice for 10 minutes. Scale up the volume if separating more cells. For example, add 100µL of pre-diluted antibody for separating 1 × 10^8 cells in 1ml of MojoSort™ Buffer. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

6 Vortex the Streptavidin conjugated Nanobeads (to resuspend) at max speed, 5 touches. Prepare dilution by adding 5 µL of the Nanobeads to 15 µL of 1X MojoSort Buffer. Add 10µL of pre-diluted Streptavidin Nanobeads. Mix well and incubate on ice for 10 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.

7 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 x 10^7	2 x 10^8	500µL for up to 10^8 cells	1ml	1 ml	1 ml
Medium Capacity	1 x 10^8	2 x 10^9	500µL for up to 10^9 cells	3ml	3 ml	5 ml
Large Capacity	1 x 10^9	2 x 10^{10}	500µL for up to 10^{10} cells	20-50ml	30 ml	20 ml

Example of magnetic separation with medium capacity columns:

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

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

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

11 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.

12 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.