Version 2

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MojoSort™ Human CD56 Nanobeads Column Protocol V.2

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1 *Works for me* This protocol is published without a DOI.

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SUBMIT TO PLOS ONE

ABSTRACT

MojoSort™ Human CD56 Nanobeads Column Protocol

EXTERNAL LINK

https://www.biolegend.com/en-us/protocols/mojosort-human-cd56-nanobeads-column-protocol

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PROTOCOL CITATION

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KEYWORDS

MojoSort, CD56, cell separation, magnetic beads, BioLegend, magnetic columns, nanobeads

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GUIDELINES

Introduction: 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.

Important Note: 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 TEXT

- MojoSort™ Buffer (5X) (Cat. No.480017)
- Adjustable pipettes
- 70 µm filters (one per sample)
- 5 mL (12 x 75mm) or 14 mL (17 x 100 mm) polypropylene tubes
- Reagents for sample preparation
- Reagents and instruments (flow cytometer) to determine yield and purity

BEFORE STARTING

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.

Protocol

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 x 75 mm) polypropylene tube.
Note: Keep MojoSort™ Buffer on ice throughout the procedure.

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

4 Aliquot 100 µL (10^7cells) into a new tube.

5 15m
Vortex the antibody-conjugated Nanobeads (to resuspend) at max speed, 5 touches, and prepare the dilutions to test. **Add 10µL of pre-dilutedconjugated 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 x 10^8cells in 1 mL of MojoSort™ Buffer. When working with less than 10^7cells, use indicated volumes for 10^7cells.

6 5m
Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes.

7 Discard the supernatant.

8 Resuspend cells in the appropriate amount of MojoSort™ Buffer and proceed to separation. At least 500 µL is needed

for column separation.

	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:

1. Place the column in a magnetic separator that fits the column.
2. Rinse the column with 3 mL of cell separation buffer.
3. Add the labeled cell suspension to the column through a 30 µm filter and collect the fraction containing the unlabeled cells.
4. 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.
5. 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.

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

Data

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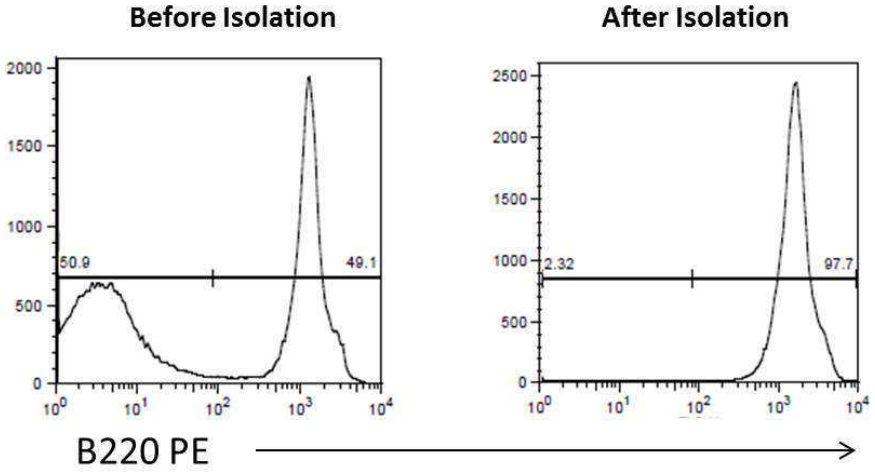
Kit	Purity	Yield
Mouse CD19 Nanobeads	97.7%	94.4%



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

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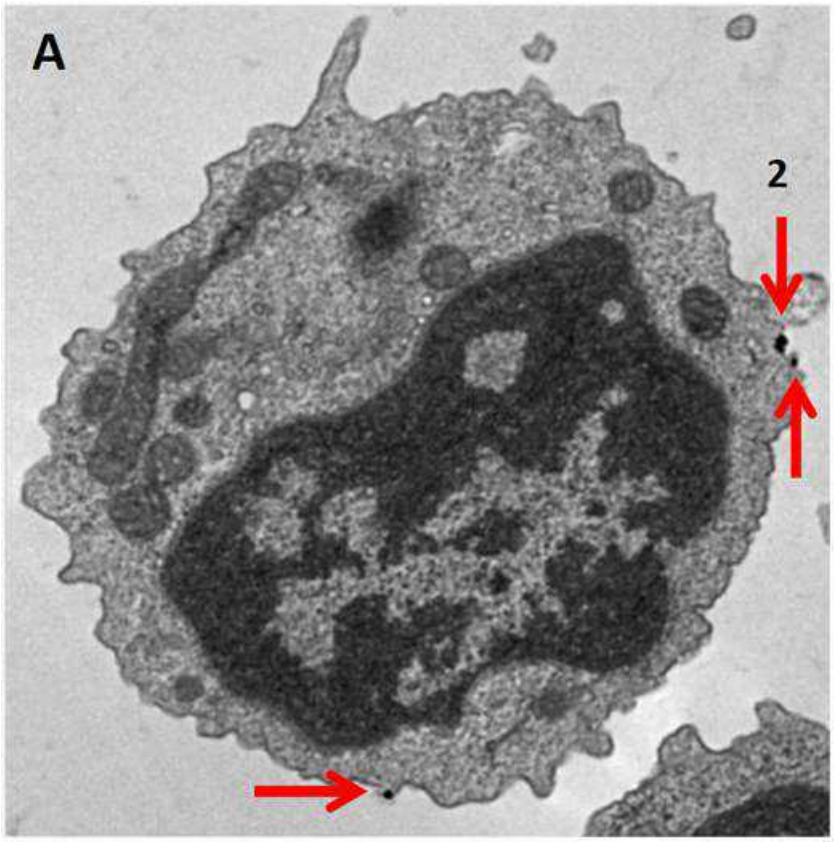

Electron Microscopy. MojoSort™ Nanobead-isolated CD19^{+} cells using columns do not display more bound beads on the cell surface (A) as compared to cells isolated with a compatible commercial product using the same columns (B). Red arrows indicate where the particles are located. Numbers indicate either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