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MojoSort™ Streptavidin Nanobeads Column Protocol - Negative Selection V.2 [↗](#)

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Works for me

[dx.doi.org/10.17504/protocols.io.693hh8n](https://doi.org/10.17504/protocols.io.693hh8n)

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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 and less antibody cocktail than with other commercial suppliers. We recommend a titration to find the best dilution factor. However, as a general rule, dilutions ranging from 1:2 to 1:10 for the antibody cocktail can be used. Dilutions ranging from 1:5 to 1:20 for the Streptavidin Nanobeads can be used. Please contact BioLegend Technical Service (tech@biolegend.com) if further assistance is needed.

EXTERNAL LINK

<https://www.biolegend.com/protocols/mojosort-streptavidin-nanobeads-column-protocol-negative-selection/4772/>

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™.

Protocol Selection: If your target cells are the labeled cells (the positive fraction), use the Streptavidin Nanobeads Column Protocol – Positive Selection. If your target cells are the unlabeled cells (negative fraction), use the Streptavidin Nanobeads Column Protocol - Negative Selection.

MATERIALS

NAME	CATALOG #	VENDOR
MojoSort™ Buffer	480017	BioLegend
MojoSort™ Streptavidin Nanobeads	View	
Biotin-Conjugated Primary Antibody	View	

MATERIALS TEXT

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 x 75 mm) polypropylene tube.
Note: Keep MojoSort™ Buffer on ice throughout the procedure. 5m
- 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. 15m
- 4 Aliquot 100 µL of cell suspension (10^7 cells) into a new tube. Check the recommended usage for flow cytometric staining of the Biotin-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 Biotin-conjugated antibody** to the cell suspension, mix well and **incubate on ice for 15 minutes**.

Note: For the Biotin-conjugated antibodies, we recommend to do a titration to determine the optimal concentration. 5m
- 5 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes. 15m
- 6 Discard the supernatant and resuspend cells in 100 µL of MojoSort™ Buffer.
- 7 Resuspend the beads by vortexing, maximum speed, 5 touches. **Add the appropriate volume of pre-diluted Streptavidin Nanobeads**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells. For example, if the volume of pre-diluted Nanobeads for 1×10^7 cells is 10 µL, add 100 µL 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 to do a titration to determine the optimal concentration. 5m
- 8 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes.
- 9 Discard the supernatant.
- 10 Resuspend the cells in 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 in at least 500 μ L of buffer to the column through a 30 μ m filter and collect the fraction containing the unlabeled cells. These are the cells of interest; do not discard.
- 14 Wash the cells in the column **2 times** with 3 mL of buffer and collect the fraction containing the untouched cells. Combine with the collected fraction from step 3.
- 15 If desired, the labeled cells can be collected by taking 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. The labeled cells may be useful as staining controls, to monitor purity/yield, or other purposes.



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