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MojoSort™ Streptavidin Nanobeads Protocol - Negative Selection

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1 Works for me dx.doi.org/10.17504/protocols.io.7u8hnzw

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ABSTRACT

Product description and procedure summary:

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

Target cells are isolated by incubating your sample with a Biotin-conjugated antibody or antibody cocktail, followed by incubation with magnetic Streptavidin Nanobeads (Cat. No. 480015/480016). The magnetically labeled fraction is retained by the use of a magnetic separator. The untouched cells are collected. These are the cells of interest; do not discard the liquid. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Note: This procedure is optimized for the isolation of 10^7 to 2 x 10^8 cells per tube. If working with fewer than 10^7 cells, keep volumes as indicated for 10^7 cells. For best results, optimize the conditions to your specific cell number and tissue. Prepare fresh MojoSort™ Buffer solution by diluting the 5X concentrate with sterile distilled water. *Scale up volumes if using 14 mL tubes and Magnet, and place the tube in the magnet for 10 minutes.*

EXTERNAL LINK

https://www.biolegend.com/protocols/mojosort-streptavidin-nanobeads-protocol-negative-selection/4749/

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™.

Application notes: To use this product in magnetic separation columns, a titration of the Nanobeads should be performed. Optimal concentration for magnetic separation columns is lot-specific. Please contact BioLegend Technical Service (tech@biolegend.com) for further assistance on how to use MojoSort™ Nanobeads in magnetic separation columns.

General Tips & FAQ

- How much of Biotinylated antibodies should I use?
- For the biotinylated antibodies, we recommend to do a titration to determine the optimal concentration. In general, if using only a single biotin-conjugated antibody (and not a cocktail), then using the flow cytometry recommended concentration may work okay as long as you are washing after antibody incubation. If you are using a cocktail or plan to remove the wash after antibody incubation, you should perform titrations and optimizations.
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- How much of Streptavidin Nanobeads should I use?
- The amount of beads to use always depends on the frequency of the target, among a few other factors. The standard volume to use is 10 µL of beads per 10^7 cells. You can benefit from titrating the beads, using 2, 5, 10, and 20 µL of beads (for example). In some cases less than 10µL of beads is enough to achieve the purity and you can save on the reagent. Please note that Streptavidin Nanobeads volume should not exceed more than 20% of the total volume.
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Note: The Antibody/Nanobead volume to add should not exceed more than 20% of the 100 µL cell suspension volume. Thus, for 100 µL of cell suspension do not add more than 20 µL of Antibody/Nanobeads. If you need to add more than 20 µL, resuspend the cells in step 3 or step 6 at a higher concentration. For example, to add 50 µL of Antibody/Nanobeads, resuspend the cells to a final concentration of 2 x 10^8 cells/mL. You can then add 50 µL of Antibody/Nanobeads to 50 µL of cells. Always keep the total volume around 100µL.

MATERIALS

NAME	CATALOG #	VENDOR
MojoSort™ Magnet	480019	BioLegend
MojoSort™ Buffer	480017	BioLegend
MojoSort™ Streptavidin Nanobeads	480015, 480016	BioLegend

MATERIALS TEXT

- Adjustable pipettes
- 70 µm filters (one per sample)
- 5mL (12 x 75 mm) or 14 mL (17 x 100 mm) polypropylene tubes polypropylene tubes
- Reagents for sample preparation
- Reagents and instruments (Flow cytometer) to determine yield and purity

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 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 x 10^8 cells/mL.

4 Aliquot 100µL 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 (see General Tips and FAQ below) of Biotin-conjugated antibody** to the cell suspension, mix well and **incubate on ice for 15 minutes.**
Optional: Take an aliquot before adding the cocktail to monitor purity and yield.

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

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

7 Resuspend the beads by vortexing, maximum speed, 5 touches.**Add the appropriate volume (see General Tips and FAQ below) of 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 Nanobeads for 1x10^7 cells is 10 µL, add 100 µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

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 Add 2.5 mL of MojoSort™ Buffer.
Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.

11 Place the tube in the magnet for 5 minutes.
Optional: Take a small aliquot before placing the tube in the magnet to monitor purity and yield. Keep unused cells to be used as control or other applications if needed.

12 Pour out the unlabeled fraction. If these are your cells of interest, **DO NOT DISCARD**. Resuspend the labeled cells in 2.5 mL MojoSort™ Buffer.

13 Repeat steps 10-12 on the labeled fraction once more for a total of **2 separations**. Pool the unlabeled fractions. The labeled cells may be useful as staining controls, to monitor purity/yield, or other purposes.
Note: Repeating the magnetic separation increases the yield, without a strong impact on the purity. The yield will typically increase about 8-10% with a second separation. The purity may decrease 1-2% with each separation.