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MojoSort™ Human CD56 Nanobeads Protocol

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

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EXTERNAL LINK

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

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MojoSort, CD56, cell separation, magnetic beads, BioLegend

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GUIDELINES

Product description and procedure summary: The cells targeted by the Nanobeads are either selected or depleted by incubating your sample with the directly conjugated magnetic particles. The magnetically labeled fraction is retained by the use of a magnetic separator. After collection of the targeted cells, downstream applications include functional assays, gene expression, phenotypic characterization, etc.

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. 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. We do not recommend using MojoSort™ particles for BD's IMag™ or Life Technologies' DynaMag™. Please contact BioLegend Technical Service (tech@biolegend.com) for more information and guidance on magnets and lot-specific information.

MATERIALS TEXT

- MojoSort™ Buffer (5X) (Cat. No.480017)
- MojoSort™ Magnet (Cat. No.480019/480020) or compatible magnetic separation system
- 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: 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 14mL tubes and Magnet, and place the tube in the magnet for 10 minutes.*

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 100 µL of cell suspension (10^7 cells) into a new tube. **Add 20µL of True-Stain Monocyte Blocker™,** 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 True-Stain Monocyte Blocker™ for 1x10^7 cells is 20µL, add 200µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

5 Resuspend the beads by vortexing, maximum speed, 5 touches. Add **10µL of Human CD56 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 Nanobeads for separating 1 x 10^8 cells in 1 mL of MojoSort™ Buffer. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

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

7 Discard the supernatant.

8 Add 2.5mL 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.

9 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.

10 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.

11 Repeat steps 9-10 on the labeled fraction twice more for a total of **3 separations**. Pool the unlabeled fractions and keep the labeled cells. The fraction that is not of interest may be useful as staining controls, to monitor purity/yield, or other purposes.

Optional: Take a small aliquot to monitor purity and yield.