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MojoSort™ Human Pan DC Isolation Kit Protocol

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol covers usage of BioLegend's MojoSort™ Human Pan DC Isolation Kit Protocol.

EXTERNAL LINK

<https://www.biolegend.com/en-us/protocols/mojosort-human-pan-dc-isolation-kit-protocol>

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PROTOCOL CITATION

Ken Lau 2021. MojoSort™ Human Pan DC Isolation Kit Protocol. **protocols.io**
<https://protocols.io/view/mojosort-human-pan-dc-isolation-kit-protocol-bszcnf2w>

KEYWORDS

MojoSort, cell separation, magnetic beads, BioLegend, dendritic cells

LICENSE

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GUIDELINES

Product description and procedure summary: Target cells are depleted by incubating your sample with the biotin 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.

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.

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 2×10^8 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.*

- 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 $300 \times g$ for 5 minutes, and resuspend in an appropriate volume of MojoSort™ Buffer. Count and adjust the cell concentration to 1×10^8 cells/mL.
- 4 Aliquot 100 µL of cell suspension (10^7 cells) into a new tube. **Add 10 µL of Human TruStain FcX™**. 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 Human TruStain FcX™ 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.
- 5 Add **10 µL of the Biotin-Antibody Cocktail**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells. For example, add 100 µL of Antibody Cocktail 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.
Optional: Take an aliquot before adding the cocktail to monitor purity and yield.
- 6 Resuspend the beads by vortexing, maximum speed, 5 touches. Add **20 µL of Streptavidin Nanobeads**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells. For example, add 200 µL of 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 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at $300 \times g$ for 5 minutes.
- 8 Discard supernatant.
- 9 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.
- 10 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.

- 11 Pour out and collect the liquid. These are your cells of interest; **DO NOT DISCARD**. Resuspend the labeled cells in 2.5 mL MojoSort™ Buffer.

