MojoSort™ Human Pan DC Isolation Kit 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

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

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https://protocols.io/view/mojosort-human-pan-dc-isolation-kit-protocol-btasniee
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KEYWORDS

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

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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.

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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 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 x *g* 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. 5m

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 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. 10m

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 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. 15m
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 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. 15m

7 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300 x *g* for 5 minutes. 5m

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. 5m

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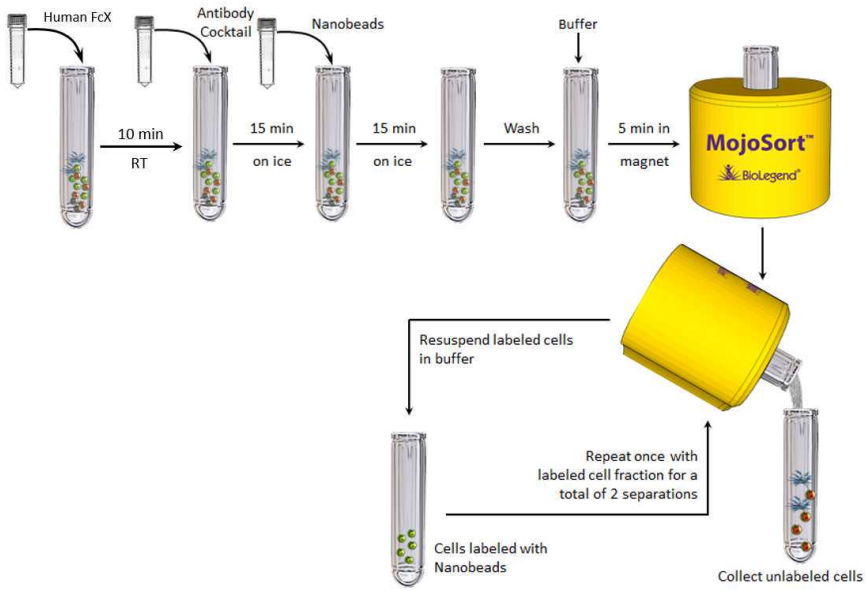
Optional: Take a small aliquot before placing the tube in the magnet to monitor purity and yield. Keep unused cells to be used as control or other applications if needed.

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.



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