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MojoSort™ Whole Blood Human Neutrophil Isolation Kit Protocol V.2

Ken Lau[1]

[1]BioLegend

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BioLegend
Tech. support email: tech@biolegend.com

Ken Lau

ABSTRACT

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

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EXTERNAL LINK

https://www.biolegend.com/en-us/protocols/mojosort-whole-blood-human-neutrophil-isolation-kit-protocol

PROTOCOL CITATION

Ken Lau 2021. MojoSort™ Whole Blood Human Neutrophil Isolation Kit Protocol. **protocols.io** https://protocols.io/view/mojosort-whole-blood-human-neutrophil-isolation-ki-bvwin7ce
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MojoSort, cell separation, magnetic beads, BioLegend, neutrophils

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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.

Note: This procedure is optimized using 1 mL whole blood per tube. Scale volumes in this procedure accordingly, e.g. using 2 mL whole blood, use twice the amount of nanobeads. 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.**

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

Important Note: 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™.

1 Collect whole blood in collection tube that has anticoagulant, preferably EDTA.
Note: Keep MojoSort™ Buffer on ice throughout the procedure.

2 15m
Aliquot 1 mL of human whole blood into a 5 mL (12 x 75 mm) polypropylene tube. **Add 10 µL of the Biotin-Antibody Cocktail**. Mix well and **incubate on ice for 15 minutes**. Scale up or down the volume accordingly if separating more blood. For example, add 20 µL of biotinylated antibody cocktail for separating 2 mL blood.

3 15m
Resuspend the beads by vortexing, maximum speed, 5 touches. Add **10 µL of Streptavidin Nanobeads**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more volume of whole blood. For example, add 20 µL of Nanobeads for separating 2 mL of whole blood.

4 5m
Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300 x *g* for 5 minutes. Remove supernatant by Pipet aid instead of pouring.

5 Add MojoSort™ Buffer, see Table 1 for volume.
Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.
Table 1. MojoSort™ Whole Blood Human CD8 Nanobeads Protocol: Separation Volumes and Magnet Times

A	B	C	D
	Cat. No.	**MojoSort™ Buffer Volume**	**Magnetic Incubation Time**
MojoSort™ magnet	480019	For samples ≤ 1 mL: Add 3mL buffer for separation	5 min
MojoSort™ 14 mL magnet	480020	For samples ranging 1 – 5 mL: Top volume up to 10mL for each separation (e.g. for 5 mL sample add 5 mL buffer for the first separation, then add 10 mL buffer for subsequent separations)	10 min

6 10m
Place the tube in the magnet, see Table 1 for magnetic incubation time.
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.

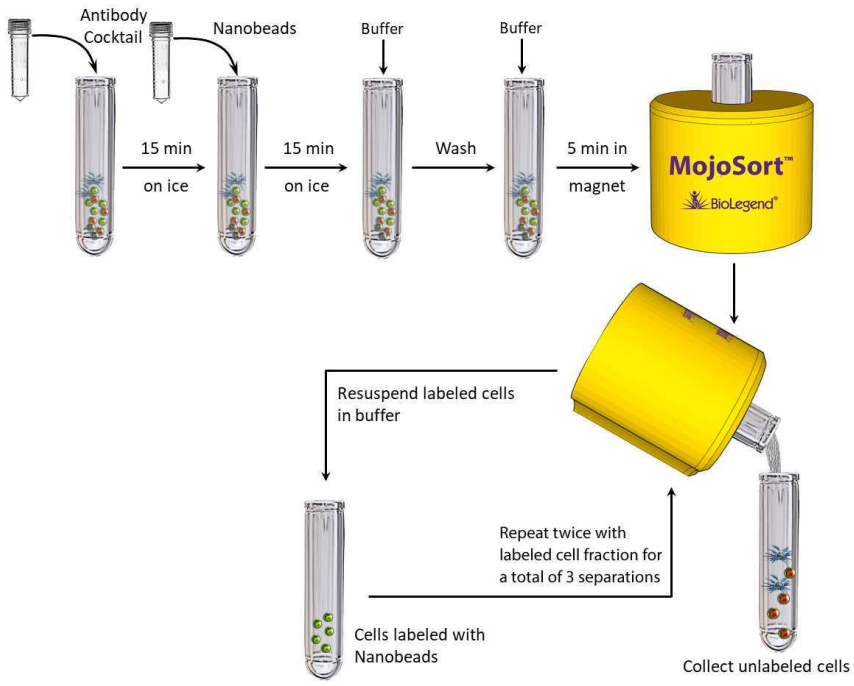
7 Pour out the unlabeled fraction. If these are your cells of interest, **DO NOT DISCARD**. Resuspend the labeled cells in MojoSort™ Buffer.

8 Repeat steps 4-6 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. 40m
Optional: Take a small aliquot to monitor purity and yield.

9 Resuspend the magnetically labeled cells in desired volume, making sure to rinse the sides of the tube for better recovery.

10 (Optional) Lyse remaining erythrocytes using 20 mL room temperature 1X Red Blood Cell Lysis Buffer (10X Cat. No. 420301/420302). Incubate 15 min, in dark at room temperature followed by filtering through a cell strainer (40-70 µm). Wash twice with FACS wash buffer. 15m

11 **Chart Protocol:**



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.