Mar 12, 2021

MojoSort™ Whole Blood Human Neutrophil Isolation Kit Column Protocol

Ken Lau[1]

[1]BioLegend

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

BioLegend
Tech. support email: tech@biolegend.com

Ken Lau

SUBMIT TO PLOS ONE

ABSTRACT

MojoSort™ Whole Blood Human Neutrophil Isolation Kit Column Protocol

EXTERNAL LINK

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

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PROTOCOL CITATION

Ken Lau 2021. MojoSort™ Whole Blood Human Neutrophil Isolation Kit Column Protocol. **protocols.io** https://protocols.io/view/mojosort-whole-blood-human-neutrophil-isolation-ki-btawnife

KEYWORDS

MojoSort, cell separation, magnetic beads, BioLegend, magnetic columns, nanobeads, neutrophils

CREATED

Mar 12, 2021

LAST MODIFIED

Mar 12, 2021

PROTOCOL INTEGER ID

48182

GUIDELINES

Introduction: BioLegend MojoSort™ nanobeads work in commonly used separation columns, based on our internal research as well as validation by external testing by academic labs. This simple protocol consists of following the MojoSort™ protocol to label the cells with **pre-diluted** MojoSort™ reagents and using the columns as indicated by the manufacturer.

Note: Due to the properties of our beads, it may be possible to use far fewer beads that with other commercial suppliers. We recommend a titration to find the best dilution factor. However, as a general rule, dilutions ranging from 1:3 to 1:20 for the Nanobeads can be used. Please contact BioLegend Technical Service (tech@biolegend.com) if further assistance is needed.

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™.

MATERIALS TEXT

- MojoSort™ Buffer (5X) (Cat. No.480017)
- Human TruStain FcX™ (Cat. No. 422301).
- 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

Protocol 55m

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 5 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 10 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 larger starting volume of whole blood. For example, add 20 µL of pre-diluted 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 15m
Proceed to separation on column as indicated by the manufacturer.
Note: There are several types of commercially available columns, depending on your application. Choose the one that fits best your experiment. *This kit requires the use of a column compatible with processing of human whole blood.*

6 5m
Column:

A	B	C	D	E
	Max. volume of whole blood	**Column rinse volume**	**Cell wash volume**	**Elution volume**
Whole Blood Column	15 mL	3 mL	3x3 mL	5 mL

Example of magnetic separation with whole blood columns:
1. Place the column in a magnetic separator that fits the column.
2. Rinse the column with 3 mL of cell separation buffer.

3. Add the labeled whole blood to the column through a 30 µm filter and collect the fraction containing the unlabeled cells.
4. Wash the cells in the column **3 times** with 3 mL of buffer and collect the fraction containing the unlabeled cells. Combine with the collected fraction from step 3. These cells may be useful as controls, to monitor purity/yield, or other purposes.
5. Take away the column from the magnet and place it on a tube. Then add 5 mL of elution buffer and flush out the magnetically labeled fraction with a plunger or supplied device. These are the positively isolated cells of interest; do not discard.
6. (*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 (40-70µM). Wash twice with FACS wash buffer.