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## MojoSort™ Human CD14+ Monocyte Isolation Kit Column Protocol V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bbwaipae

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### ABSTRACT

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 and less antibody cocktail than with other commercial suppliers. We recommend a titration to find the best dilution factor. However, as a general rule, dilutions ranging from 1:2 to 1:10 for the antibody cocktail can be used. Dilutions ranging from 1:5 to 1:20 for the Streptavidin Nanobeads can be used. Please contact BioLegend Technical Service (tech@biolegend.com) if further assistance is needed.

### EXTERNAL LINK

<https://www.biolegend.com/protocols/mojosort-human-cd14-monocyte-isolation-kit-column-protocol/4764/>

### GUIDELINES

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™.

**Sample Preparation:** It is strongly recommended that platelets be removed prior to the isolation of monocytes using a suitable method. See recommended platelet removal protocol below.

### MATERIALS

NAME	CATALOG #	VENDOR
Human TruStain FcX™	422301	BioLegend
MojoSort™ Buffer	480017	BioLegend

### MATERIALS TEXT

Additional reagents:

- commercially available cell separation columns
- 5 mL polypropylene tubes
- 70 µm cell strainer

This protocol works with the following MojoSort™ Kits (cat#):  
480047, 480048

### Platelet Removal Protocol

- 1 Dilute blood with 2-4 times (volume/volume) 1X PBS.

- 2 Carefully layer diluted blood over 12.5 mL of isolation medium in a 50mL tube.
- 3 Centrifuge at 400xg for 25 minutes at room temperature in a swinging-bucket rotor without the brake. 25m
- 4 Aspirate the upper layer of the gradient (serum), leaving the interphase containing the mononuclear cells undisturbed.
- 5 Carefully transfer the mononuclear cells to a new 50 mL tube.
- 6 Fill the tube with 1X PBS, mix, and centrifuge at 200xg for 8 minutes at room temperature. Carefully remove supernatant as much as possible. 8m
- 7 Repeat step 6.
- 8 Proceed to separation protocol.

#### Separation Protocol

- 9 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.
- 10 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  $1 \times 10^8$  cells/mL. 5m
- 11 Aliquot 100 µL of cell suspension ( $10^7$  cells) into a new tube. **Add 5 µL of Human TruStain FcX™ (Fc Receptor Blocking Solution)**, 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  $1 \times 10^7$  cells is 5 µL, add 50 µL for  $1 \times 10^8$  cells. When working with less than  $10^7$  cells, use indicated volumes for  $10^7$  cells. 10m
- 12 **Add 10 µL of the pre-diluted 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  $1 \times 10^8$  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.* 15m
- 13 Resuspend the beads by vortexing, maximum speed, 5 touches. **Add 10 µL of pre-diluted Streptavidin 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  $1 \times 10^8$  cells in 1 ml of MojoSort™ Buffer. When working with less than  $10^7$  cells, use indicated volumes for  $10^7$  cells. 15m
- 14 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes. 5m
- 15 Discard supernatant.

- 16 Resuspend cells in the appropriate amount of MojoSort™ Buffer and proceed to separation. At least 500 µL is needed for column separation.

**Note:** There are several types of commercially available columns, depending on your application. Choose the one that fits best your experiment:

	Max. number of labeled cells	Max. number of total cells	Cell suspension volume	Column rinse volume	Cell wash volume	Elution volume
<b>Small Capacity</b>	$1 \times 10^7$	$2 \times 10^8$	500µL for up to $10^8$ cells	1ml	1 ml	1 ml
<b>Medium Capacity</b>	$1 \times 10^8$	$2 \times 10^9$	500µL for up to $10^9$ cells	3ml	3 ml	5 ml
<b>Large Capacity</b>	$1 \times 10^9$	$2 \times 10^{10}$	500µL for up to $10^{10}$ cells	20-50ml	30 ml	20 ml

Example of magnetic separation with medium capacity columns:

- 17 Place the column in a magnetic separator that fits the column.
- 18 Rinse the column with 3 mL of cell separation buffer.
- 19 Add the labeled cell suspension in at least 500 µL of buffer to the column through a 30 µm filter and collect the fraction containing the unlabeled cells. These are the cells of interest; do not discard.
- 20 Wash the cells in the column **2 times** with 3 mL of buffer and collect the fraction containing the untouched cells. Combine with the collected fraction from step 3.
- 21 If desired, the labeled cells can be collected by taking away the column from the magnet and place it on a tube. Then add 5 mL of buffer and flush out the magnetically labeled fraction with a plunger or supplied device. The labeled cells may be useful as staining controls, to monitor purity/yield, or other purposes.



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