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

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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 that 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.

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

MATERIALS

⊗ Human TruStain FcX™ BioLegend Catalog #422301

⊗ MojoSort™ Buffer BioLegend Catalog #480017

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 1×10^8 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 1×10^7 cells is 5 µL, add 50 µL for 1×10^8 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 1×10^8 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 1×10^8 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
Small Capacity	1×10^7	2×10^8	500µL for up to 10^8 cells	1ml	1 ml	1 ml
Medium Capacity	1×10^8	2×10^9	500µL for up to 10^9 cells	3ml	3 ml	5 ml
Large Capacity	1×10^9	2×10^{10}	500µL for up to 10^{10} cells	20-50ml	30 ml	20 ml

Example of magnetic separation with medium capacity colu..

- 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.