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MojoSort™ Human anti-APC Nanobeads Column Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.7afhibn

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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 than 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.

EXTERNAL LINK

https://www.biolegend.com/protocols/mojosort-human-anti-apc-nanobeads-column-protocol/4758/

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™.

MATERIALS

NAME	CATALOG #	VENDOR
Human TruStain FcX™	422301	BioLegend
MojoSort™ Buffer	480017	BioLegend
MojoSort™ Human anti-APC Nanobeads	480089	BioLegend
APC-Conjugated Anti-Human Antibody	View	BioLegend

MATERIALS TEXT

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

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.

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5m

3 Filter the cells with a 70 µm cell strainer, centrifuge at 300xg for 5 minutes, and resuspend in a small volume of MojoSort™ Buffer. Count and adjust the cell concentration to 1 x 10^8 cells/mL by adding MojoSort™ Buffer.

1m

4 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, add 50 µL of Human TruStain FcX™ 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

5 Check the recommended usage for flow cytometric staining of the APC-conjugated antibody indicated in the antibody technical datasheet. Calculate the volume to stain 10^7 cells (or desired amount of cells). **Add the appropriate volume of pre-diluted APC-conjugated antibody** to the cell suspension, mix well and **incubate on ice for 15 minutes.**
Note: For the APC-conjugated antibodies, we recommend to do a titration to determine the optimal concentration

5m

6 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes.

7 Discard the supernatant and resuspend cells in 100 µL of MojoSort™ Buffer.

15m

8 Vortex the anti-APC Nanobeads (to resuspend) at max speed, 5 touches, and prepare the dilutions to test. **Add the appropriate volume of pre-diluted anti-APC Nanobeads**. Mix well and **incubate on ice for 15 minutes.** Scale up the volume accordingly if separating more cells. For example, if the volume of pre-diluted Nanobeads for 1x10^7 cells is 10 µL, add 100 µL of pre-diluted 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.
Note: The amount of Nanobeads to use always depends on the frequency of the target, among a few other factors. We recommend to do a titration to determine the optimal concentration.

5m

9 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes.

10 Discard the supernatant.

11 Add 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 x 10^7	2 x 10^8	500µL for up to 10^8 cells	1ml	1 ml	1 ml
Medium Capacity	1 x 10^8	2 x 10^9	500µL for up to 10^9 cells	3ml	3 ml	5 ml
Large Capacity	1 x 10^9	2 x 10^{10}	500µL for up to 10^{10} cells	20-50ml	30 ml	20 ml

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Example of magnetic separation with medium capacity columns:

12 Place the column in a magnetic separator that fits the column.

13 Rinse the column with 3 mL of cell separation buffer.

14 Add the labeled cell suspension to the column through a 30 µm filter and collect the fraction containing the unlabeled cells.

15 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.

16 Take 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. These are the positively isolated cells of interest; do not discard. To increase the purity of the magnetically labeled fraction repeat the isolation process with a new, freshly prepared column.

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