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MojoSort™ Human anti-APC Nanobeads Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.7x2hpqe

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ABSTRACT

Product description and procedure summary:
Target cells are positively selected or depleted by incubating the sample with an anti-human APC conjugated antibody, followed by incubation with magnetic anti-APC Nanobeads. The magnetically labeled fraction is retained by the use of a magnetic separator. These are the APC+ cells, do not discard them if those are the cells of interest. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Note: This procedure is optimized for the isolation of 10^7 to 2 x 10^8 cells per tube. If working with fewer than 10^7 cells, keep volumes as indicated for 10^7 cells. For best results, optimize the conditions to your specific cell number and tissue. 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.*

EXTERNAL LINK

https://www.biolegend.com/protocols/mojosort-human-anti-apc-nanobeads-protocol/4755/

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™.

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.

MATERIALS

NAME	CATALOG #	VENDOR
MojoSort™ Magnet	480019	BioLegend
MojoSort™ Human anti-APC Nanobeads	480089	BioLegend
MojoSort™ Buffer	480017	BioLegend

MATERIALS TEXT

- Adjustable pipettes
- 70µm filters (one per sample)
- 5mL (12 x 75mm) or 14mL (17 x 100mm) polypropylene tubes
- Reagents for sample preparation
- Reagents and instruments (Flow cytometer) to determine yield and purity

1 Prepare cells from your tissue of interest. Kits for human samples have been optimized for PBMCs, please prepare the cells using a suitable method.

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.

3 Filter the cells with a 70 µm cell strainer, cenrifuge 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.

4 Aliquot 100µL of cell suspension (10^7 cells) into a new tube. **Add 5µL 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 1x10^7 cells is 5 µL, add 50 µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

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 APC-conjugated antibody** to the cell suspension, mix well and **incubate on ice for 15 minutes**.

Optional: Take an aliquot before adding the antibody to monitor purity and yield.

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

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

8 Resuspend the beads by vortexing, maximum speed, 5 touches. **Add the appropriate volume of Human anti-APC Nanobeads** to the cell suspension, mix well and **incubate on ice for 15 minutes**. The volume of MojoSort™ Human anti-APC Nanobeads should be adjusted depending on starting percentage of cell type to be isolated. For 1x10^7 cells in 100 µl of buffer, use the following volumes:

Cell Type	Cell Frequency in PBMCs	Volume of MojoSort™ anti-APC Nanobeads (for 1x10^7 cells in 100 µl of buffer)
CD3	50-60%	20 µL
CD4 (including monocytes)	50-55%	20 µL
CD8a (including low expressing cells)	15-20%	10 µL
CD19	5-10%	10 µL
TCR Vδ2	1-3%	5 µL

Scale up the volume accordingly if separating more cells. For example, if the volume of Nanobeads for 1x10^7 cells is 10 µL, add 100 µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

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 2.5 mL of MojoSort™ Buffer.
Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.

12 Place the tube in the magnet for 5 minutes.
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.

13 Pour out the unlabeled fraction. If these are your cells of interest, **DO NOT DISCARD**. Resuspend the labeled cells in 2.5 mL MojoSort™ Buffer.

14 Repeat steps 11-13 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.
Optional: Take a small aliquot to monitor purity and yield.