MojoSort™ Isolation Kits Protocol - 1 V.2

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Abstract

Product description and procedure summary:
Target cells are depleted by incubating the sample with the biotin antibody cocktail followed by incubation with magnetic Streptavidin Nanobeads (Cat. No.480015/480016). The magnetically labeled fraction is retained by the use of a magnetic separator. The untouched cells are collected. These are the cells of interest; do not discard the liquid. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Note: This protocol has been optimized to remove washing steps after antibody cocktail and nanobeads incubations, resulting in a shorter and more convenient protocol. This procedure is optimized for the isolation of 10^7 to 2 × 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.**

Guidelines

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™.

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

MATERIALS

- MojoSort™ Buffer **BioLegend Catalog #**480017
- MojoSort™ Magnet **BioLegend Catalog #**480019
- MojoSort™ Mouse CD3 T Cell Isolation Kit **BioLegend Catalog #**480023, 480024, 480031
- MojoSort™ Mouse CD4 T Cell Isolation Kit **BioLegend Catalog #**480005, 480006, 480033
- MojoSort™ Mouse CD8 T Cell Isolation Kit **BioLegend Catalog #**480007, 480008, 480035
- MojoSort™ Human CD4 T Cell Isolation Kit **BioLegend Catalog #**480009, 480010
- MojoSort™ Human CD3 T Cell Isolation Kit **BioLegend Catalog #**480021, 480022
- MojoSort™ Mouse CD4 Naïve T Cell Isolation Kit **BioLegend Catalog #**480039, 480040
- MojoSort™ Human CD4 Naïve T Cell Isolation Kit **BioLegend Catalog #**480041, 480042
- MojoSort™ Mouse CD8 Naïve T Cell Isolation Kit **BioLegend Catalog #**480043, 480044
- MojoSort™ Human CD8 Naïve T Cell Isolation Kit **BioLegend Catalog #**480045, 480046
- MojoSort™ Mouse Pan B Cell Isolation Kit **BioLegend Catalog #**480051, 480052
- MojoSort™ Human B Cell (CD43-) Isolation Kit **BioLegend Catalog #**480061, 480062
- MojoSort™ Human CD4 Memory T Cell Isolation Kit **BioLegend Catalog #**480063, 480064
- MojoSort™ Human Naïve B Cell Isolation Kit **BioLegend Catalog #**480067, 480068
- MojoSort™ Human Pan B Cell Isolation Kit **BioLegend Catalog #**480081, 480082
- MojoSort™ Mouse Pan B Cell Isolation Kit II **BioLegend Catalog #**480087, 480088

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

Troubleshooting

1 Prepare cells from your tissue of interest or blood without lysing erythrocytes. 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 4mL in a 5 mL (12 × 75 mm) polypropylene tube.
Note: Keep MojoSort™ Buffer on ice throughout the procedure.

3 Filter the cells with a 70µm 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.

4 Aliquot 100µL of cell suspension (10^7 cells) into a new tube. **Add 10µL of the Biotin-Antibody Cocktail**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells. For example, add 100µL of biotinylated 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.

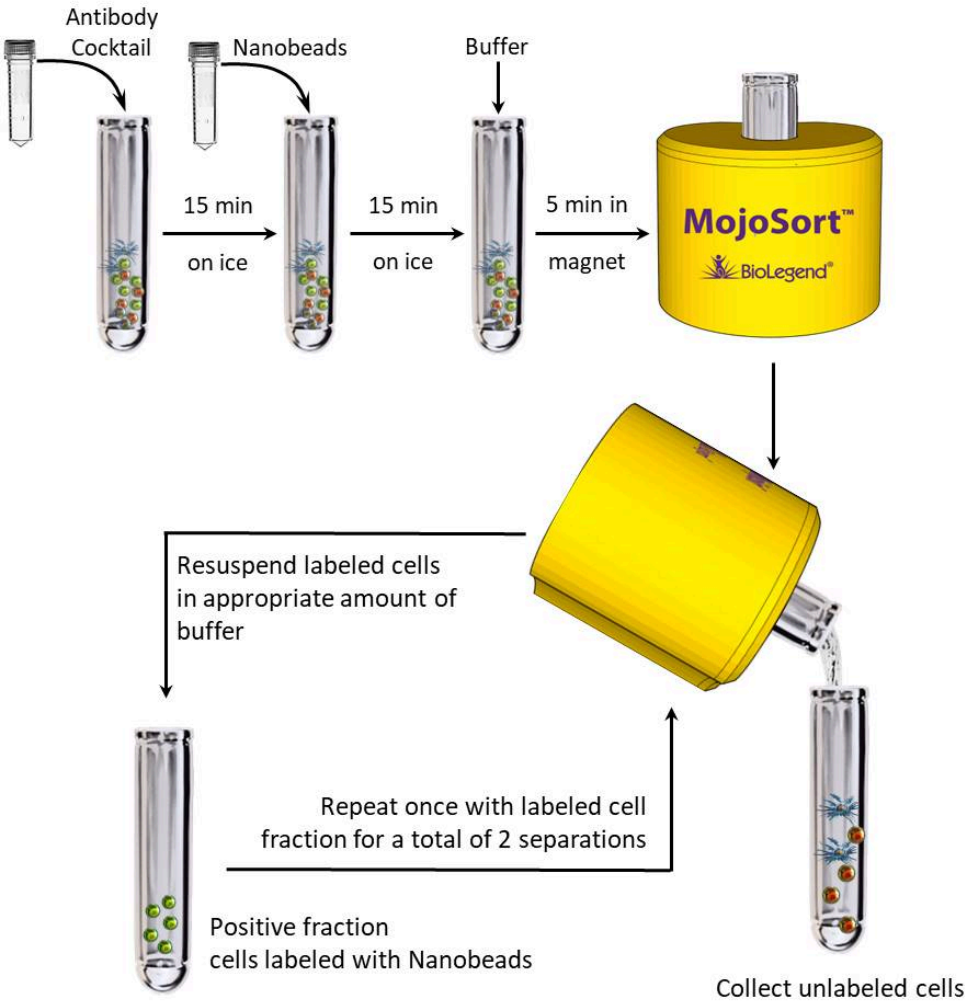
5 Resuspend the beads by vortexing, maximum speed, 5 touches. **Add 10µL of Streptavidin Nanobeads**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells. For example, add 100µL of Nanobeads for separating 1×10^8 cells in 1 ml of MojoSort™ Buffer. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

6 Add 2.5mL 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.

7 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.

8 Pour out and collect the liquid. These are your cells of interest; **DO NOT DISCARD.**

9 Repeat steps 6-8 with labeled cells once more for a total of **2 separations**. Pool the unlabeled fractions. The labeled cells may be useful as staining controls, to monitor purity/yield, or other purposes.
Note: Repeating the magnetic separation increases the yield, without a strong impact on the purity. The yield will typically increase about 8-10% with a second separation. The purity may decrease 1-2% with each separation.


Antibody Cocktail
Nanobeads
Buffer
15 min on ice
15 min on ice
5 min in magnet
MojoSort™
BioLegend®
Resuspend labeled cells in appropriate amount of buffer
Repeat once with labeled cell fraction for a total of 2 separations
Positive fraction cells labeled with Nanobeads
Collect unlabeled cells