



MojoSort™ Selection 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 14mL tubes and Magnet, and place the tube in the magnet for 10 minutes.**

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

MATERIALS

✕ MojoSort™ Magnet **BioLegend Catalog #480019**

✕ MojoSort™ Buffer **BioLegend Catalog #480017**

✕ MojoSort™ Human CD14 Selection Kit **BioLegend Catalog #480025, 480026**

✕ MojoSort™ Mouse CD3 Selection Kit **BioLegend Catalog #480099, 480100**

- 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



- 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 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.
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 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.
- 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 the unlabeled fraction. If these are your cells of interest, **DO NOT DISCARD.** Resuspend the labeled cells in MojoSort™ Buffer.
- 9 Repeat steps 6-8 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.

