

Technical Protocols

MojoSort™ Isolation Kits Protocol - 1

Reagent List

- MojoSort™ Buffer (5X) (Cat. No. [480017](#))
- MojoSort™ Magnet (Cat. No. [480019/480020](#)) or compatible magnetic separation system
- 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

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™.

Protocol

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.**

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 4 mL in a 5 mL (12 x 75 mm) polypropylene tube.

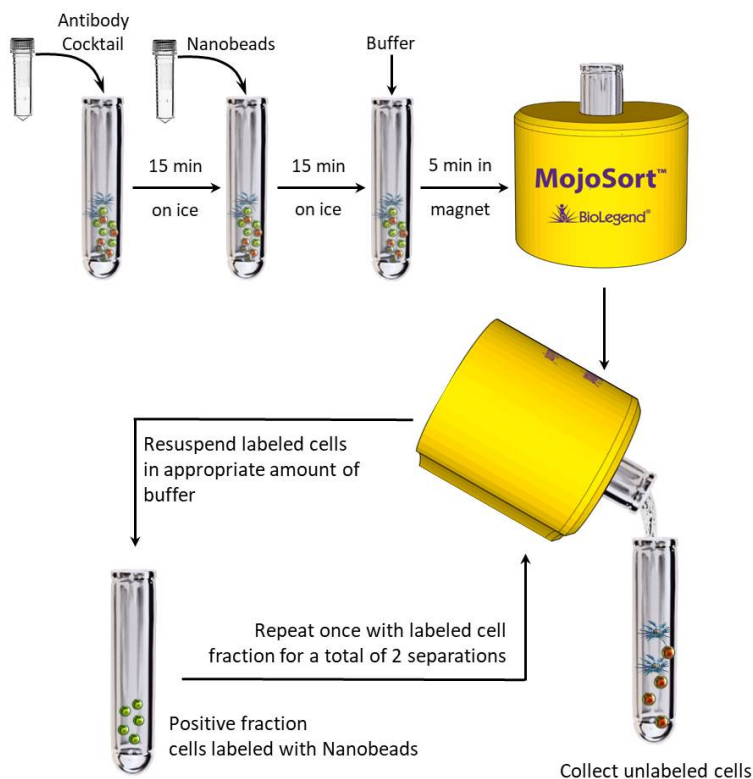
Note: For MojoSort™ Mouse CD4 Naïve T Cell Isolation Kit (480039, 480040), we recommend to use PBS without Ca/Mg in the final wash of your sample preparation, then resuspend the cells in PBS without Ca/Mg at an appropriate concentration (e.g. 1×10^8 cells/mL) and incubate on ice for 30 minutes.

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 100 μ L of cell suspension (10^7 cells) into a new tube. **Add 10 μ L 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 μ L 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 10 μ L 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 μ L 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.

Chart Protocol:



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.

Previously Viewed Products

Keratin 5 Polyclonal Antibody,
Purified

Clone: Poly19055

Purified anti-Keratin 1
Antibody

Clone: Poly19056

Clear

Purified anti-IRF1 Antibody

Clone: 13H3A44

Alexa Fluor® 488 anti-human
CD54 Antibody

Clone: HCD54

