MojoSort™ Human Pan Monocyte Isolation Kit Protocol

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 Steps

Product description and procedure summary: This kit is designed for the isolation of untouched pan monocytes from PBMC. Target cells are depleted by incubating your sample with the biotin antibody cocktail followed by incubation with magnetic Streptavidin Nanobeads. 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 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 14mL tubes and Magnet, and place the tube in the magnet for 10 minutes.*

Sample Preparation: It is strongly recommended that platelets be removed prior to the isolation of monocytes using a suitable method. See recommended platelet removal protocol below

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Platelet Removal Protocol

1. Dilute blood with 2-4 times (volume/volume) 1X PBS.
2. Carefully layer diluted blood over 12.5mL of isolation medium in a 50mL tube.
3. Centrifuge at 400xg for 25 minutes at room temperature in a swinging-bucket rotor without the brake.
4. Aspirate the upper layer of the gradient (serum), leaving the interphase containing the mononuclear cells undisturbed.
5. Carefully transfer the mononuclear cells to a new 50mL tube.
6. Fill the tube with 1X PBS, mix, and centrifuge at 200xg for 8 minutes at room temperature. Carefully remove supernatant as much as possible.
7. Repeat step 6.
8. Proceed to separation protocol.

Separation Protocol

1. In the final wash of your sample preparation, resuspend the cells in MojoSort™ Buffer by adding up to 4mL in a 5 mL (12 x 75 mm) polypropylene tube.
   Note: Keep MojoSort™ Buffer on ice throughout the procedure.
2. 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 x 10^8 cells/mL.
3. Aliquot 100 µL 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 5µL, add 50µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.
4. 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 Antibody Cocktail 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.

   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 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.
6. Wash the cells by adding MojoSort™ Buffer up to 4mL. Centrifuge the cells at 300xg for 5 minutes.
7. Discard supernatant.
8. Add 2.5mL of MojoSort™ Buffer.
   Note: If you observe aggregates, filter the suspension. To maximize yield, you can

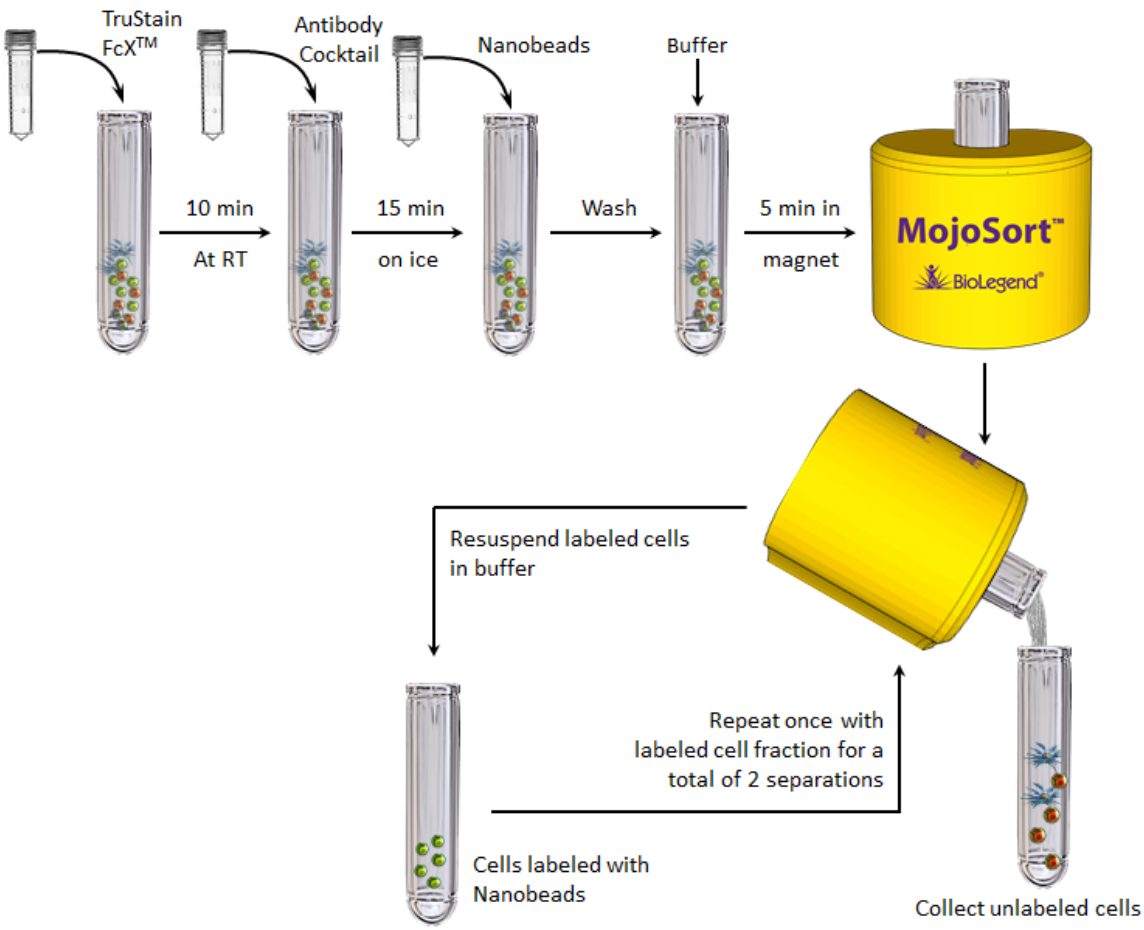
disrupt the aggregates by pipetting the solution up and down.

9. 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.

10. Pour out and collect the liquid. These are your cells of interest; **DO NOT DISCARD**. Resuspend the labeled cells in 2.5mL MojoSort™ Buffer.

11. Repeat steps 8-10 on the labeled fraction 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 Clear

Keratin 5 Polyclonal Antibody, Purified
Clone: Poly19055

Purified anti-Keratin 1 Antibody
Clone: Poly19056

Purified anti-IRF1 Antibody
Clone: 13H3A44

Alexa Fluor® 488 anti-human CD54 Antibody
Clone: HCD54