MojoSort™ Human CD4 T Cell Selection Protocol V.2

DOI
dx.doi.org/10.17504/protocols.io.7z8hp9w

Sam Li[1]

[1]BioLegend

BioLegend
Tech. support email: **tech@biolegend.com**

Sam Li
BioLegend

DOI: dx.doi.org/10.17504/protocols.io.7z8hp9w

External link: https://www.biolegend.com/protocols/mojosort-human-cd4-t-cell-selection-protocol/4279/

Protocol Citation: Sam Li . MojoSort™ Human CD4 T Cell Selection Protocol. **protocols.io https://dx.doi.org/10.17504/protocols.io.7z8hp9w**

Created: October 08, 2019

Last Modified: October 08, 2019

Protocol Integer ID: 28448

Abstract

Product description and procedure summary:
This kit is designed for the sequential positive selection of $CD4^+$ T cells from human peripheral blood mononuclear cells (PBMCs). Human monocytes express both CD14 and CD4. When using only CD4 Nanobeads for positive selection of human CD4 T cells, monocytes could be isolated along with the T cells. If this monocyte fraction does not impact your application, there is no need to address it. However, not including this population may be required. Thus, the first step in this kit is the depletion of $CD14^+$ cells using a combination of biotin anti-human CD14 and Streptavidin Nanobeads. The second step is the positive selection of the CD4 T cells using directly conjugated CD4 Nanobeads. After collection of the targeted cells, downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Note: 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 CD4 T Cell Selection Kit **BioLegend Catalog #**480038

- 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 4 mL 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, cenrifuge at 300xg for 5 minutes, and resuspend in a small volume of MojoSort™ Buffer. Count and adjust the cell concentration to 1×10^8 cells/mL by adding MojoSort™ Buffer.

4 Aliquot 100µL of cell suspension (10^7 cells) into a new tube. **Add 5µL of the biotin anti-human CD14 antibody**, mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells. For example, add 50µL for 1×10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.
Optional: Take an aliquot before adding the antibody to monitor purity and yield.

5 Resuspend the Streptavidin Nanobeads 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 for 1×10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

6 Resuspend the cells in 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.

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 in a new tube. This fraction contains the $CD4^+$ T Cells; **DO NOT DISCARD.**

9 Repeat steps 6-8 on the labeled fraction twice more, for a total of **3 separations**. Pool the unlabeled fractions containing the $CD4^+$ T Cells and keep the labeled cells. The $CD14^+$cells may be useful as staining controls, to monitor purity/yield, or other purposes.
Optional: Take a small aliquot to monitor purity and yield.

10 Centrifuge the pooled unlabeled $CD4^+$T Cells at 300xg for 5 minutes, discard supernatant, and resuspend in an appropriate volume of MojoSort™ Buffer. Count and adjust the cell concentration to 1×10^8 cells/mL.

11 Aliquot 100µL (10^7 cells), or desired amount of cells, into a new tube.

12 Resuspend the CD4 Nanobeads by vortexing, maximum speed, 5 touches. **Add 10µL of CD4 Nanobeads,**mix well and **incubate on ice for 15 minutes.** Scale up the volume accordingly if separating more cells; for example, add 100µL for 1×10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

13 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.

14 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.

15 Pour out and collect the liquid. Resuspend the labeled cells in 2.5mL MojoSort™ Buffer, these are the $CD4^+$ T Cells.

16 Repeat steps 13-15 on the labeled fraction twice more, for a total of **3 separations**. Pool the unlabeled fractions and keep the labeled $CD4^+$ T Cells. The unlabeled fraction may be useful as staining controls, to monitor purity/yield, or other purposes.
Optional: Take a small aliquot to monitor purity and yield.