MojoSort™ Human CD4 T Cell Selection Protocol

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Abstract

This protocol has been optimized to remove washing steps after antibody and nanobeads incubation, resulting in a shorter and more convenient protocol. This procedure is optimized for the isolation of 107 to 2 x 108 cells per tube. If working with fewer cells keep volumes as indicated for 107 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.

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.

Citation: Kelsey Miller MojoSort™ Human CD4 T Cell Selection Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.e3gbgjw
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Guidelines

Reagents and instruments required:

MojoSort™ Buffer (5X) (Cat. No. 480017)

MojoSort™ Magnet (Cat. No. 480019) or compatible magnetic separation system

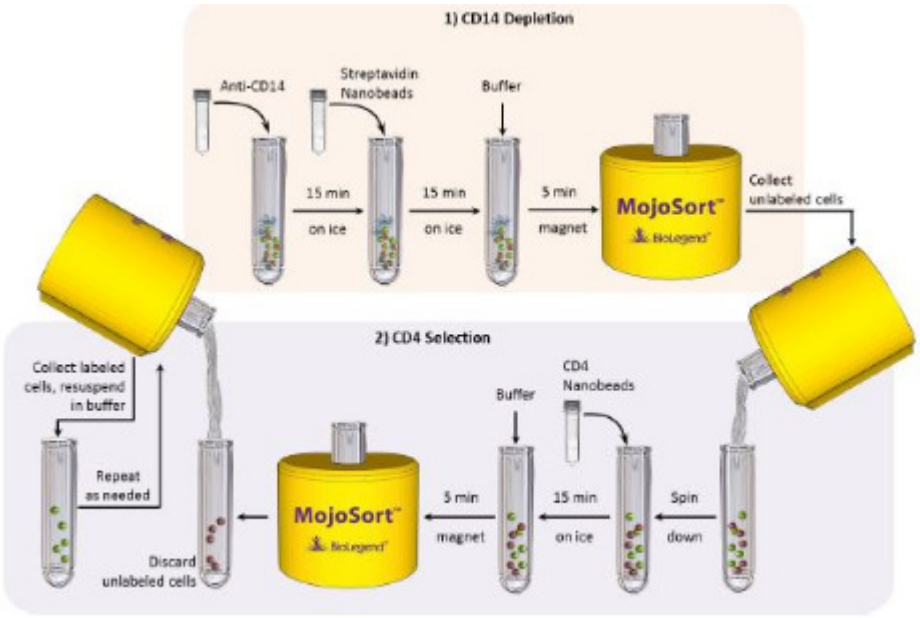
Adjustable pipettes

70 µm filters (one per sample)

5 mL (12 x 75 mm) polystyrene tubes

Reagents for sample preparation

Reagents and instruments (Flow cytometer) to determine yield and purity



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.

Protocol

Step 1.
Prepare cells from your tissue of interest without lysing erythrocytes.

Step 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 x 75 mm) polystyrene tube.

Note: Keep MojoSort™ Buffer on ice throughout the procedure.

Step 3.

Filter the cells with a 70 µm cell strainer, centrifuge at 300 x g for 5 minutes, and resuspend in anappropriate volume of MojoSort™ Buffer. Count and adjust the cell concentration to 1 x 10^8 cells/mL.

DURATION
00:05:00

Step 4.

Aliquot 100 µL of cell suspension (10^7 cells) into a new tube. Add 5 µL of the biotin anti-human CD14 antibody, mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separatingmore cells. For example, add 50 µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicatedvolumes for 10^7 cells.

Optional: Take an aliquot before adding the antibody to monitor purity and yield.

DURATION
00:15:00

Step 5.

Resuspend the Streptavidin Nanobeads by vortexing, maximum speed, 5 touches. Without washing, add 10 µL of Nanobeads, mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells; for example, add 100 µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

DURATION
00:15:00

Step 6.

Resuspend the cells in 3 mL of MojoSort™ Buffer.

Step 7.

Place the tube in the magnet for 5 minutes.

DURATION
00:05:00

Step 8.

Collect the liquid in a new tube. This fraction contains the CD4+ T Cells; DO NOT DISCARD. Centrifuge at 300 x g for 5 minutes, discard supernatant. Resuspend by flicking or in 100 uL of MojoSort™ buffer.

Step 9.

Resuspend the CD4 Nanobeads by vortexing, maximum speed, 5 touches. Add 10 µL of Nanobeads, mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells; for example, add 100 µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.10. Add 3 mL of MojoSort™ Buffer.

Step 10.

Place the tube in the magnet for 5 minutes.

DURATION
00:05:00

Step 11.

Pour out the liquid. Recover the tube and resuspend the cells in appropriate amount of buffer. These are the CD4+ T Cells.

Step 12.

Repeat steps 10 – 12 on the labeled fraction 2 more times, for a total of 3 magnetic separations.

Optional: Take a small aliquot to monitor purity and yield.