MojoSort™ Human CD14 Selection Kit Protocol

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Abstract

Product description and procedure summary:

Target cells are either selected or depleted by incubating your sample with the biotin anti-human CD14 antibody (clone 63D3) followed by incubation with magnetic Streptavidin Nanobeads. The magnetically labeled fraction is retained by the use of a magnetic separator. These are the CD14+ cells, do not discard them if those are your cells of interest. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Citation: Kelsey Miller MojoSort™ Human CD14 Selection Kit Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.e3jbgkn
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Guidelines

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 10^7 to 2 x 10^8 cells per tube from human peripheral blood mononuclear cells (PBMCs). 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.

Reagents and instruments required:

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

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

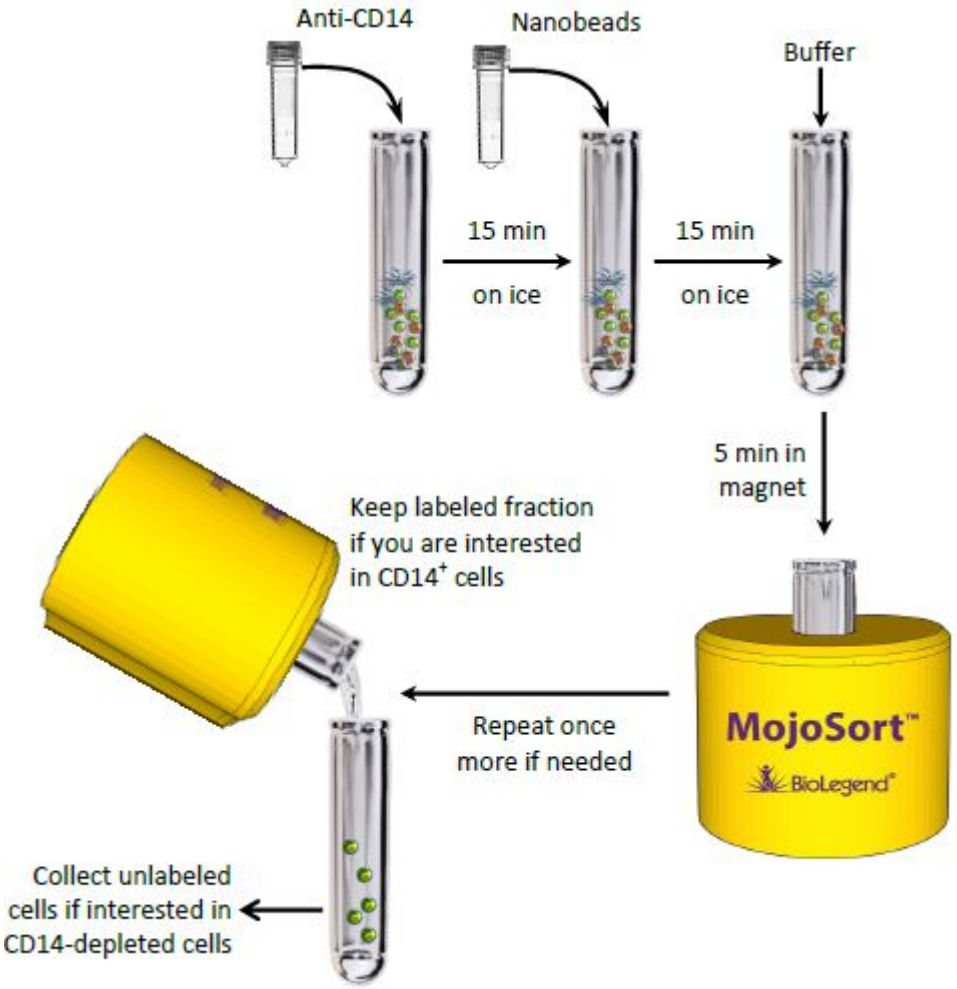
Adjustable pipettes

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 cocktail/beads 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 108 cells/mL.

Step 4.

Aliquot 100 µL of cell suspension (107 cells) into a new tube. Add 10 µL of the biotin anti-human CD14,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 108 cells. When working with less than 107 cells, use indicated volumesfor 107 cells.

Optional: Keep unused cells, or take an aliquot before adding the cocktail to monitor purity and yield.

Step 5.

Resuspend the beads by vortexing, maximum speed, 5 touches. Without washing, add 10 µL ofStreptavidin 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.

Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.

Step 7.

Place the tube in the magnet for 5 minutes.

DURATION
00:05:00

Step 8.

Pour out and collect the liquid. These are your cells of interest; **DO NOT DISCARD.**

Step 9.

If needed, add 3 mL of MojoSort™ Buffer and repeat steps 10 and 11 with the magnetically labeledfraction up to two times, and then pool the unlabeled fractions.

Note: Repeating the magnetic separation increases the yield, without a strong impact on the purity. Theyield will typically increase about 8 – 10% with a second separation, and about 2 – 5% with a thirdseparation. The purity may decrease 1 – 2% with each separation. Optional: Take a small aliquot beforeplacing the tube in the magnet to monitor purity and yield.