MojoSort™ Human CD45 Nanobeads Protocol A

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Abstract

Note: If the percentage of CD45+ cells in your sample is less than 50%, please follow Protocol A. If it is higher

than 50% then please follow protocol B.

The cells targeted by the Nanobeads are either selected or depleted by incubating your sample with the directly conjugated magnetic particles. The magnetically labeled fraction is retained by the use of a magnetic separator. After collection of the targeted cells, downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Citation: Kelsey Miller MojoSort™ Human CD45 Nanobeads Protocol A. **protocols.io**
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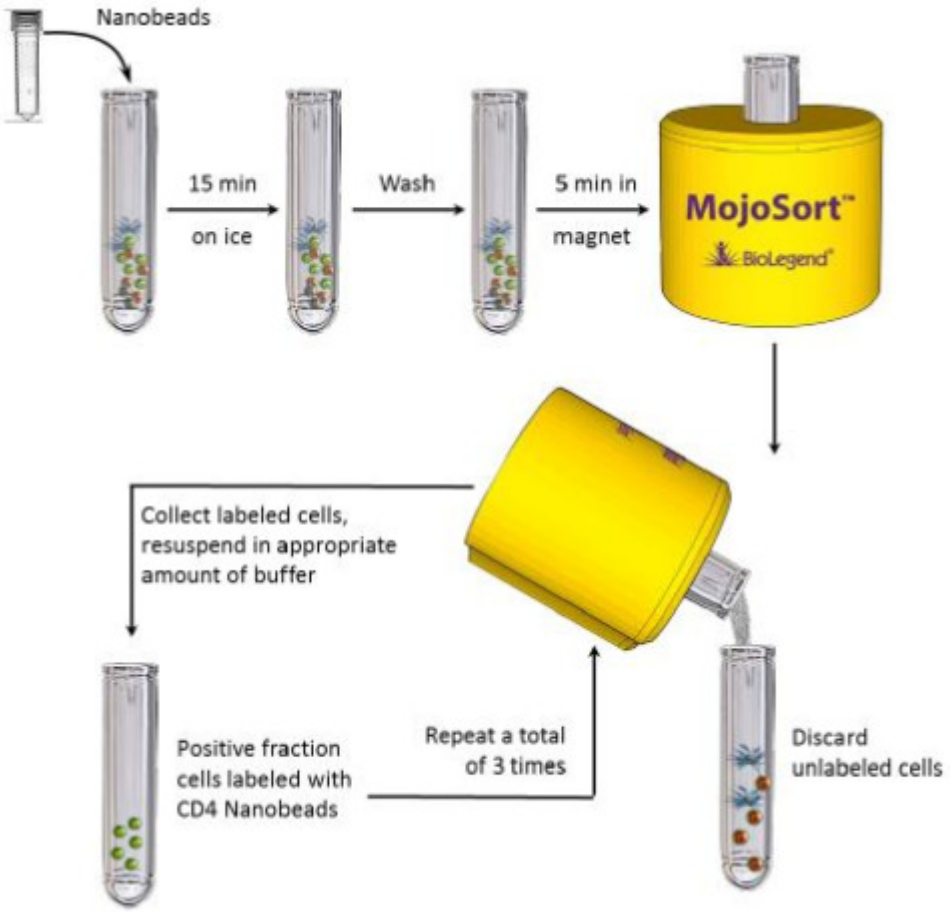
Guidelines

Protocol: 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.

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



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 4 mL 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 an appropriate 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.

Step 5.

Resuspend the beads 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.

DURATION
00:15:00

Step 6.

Add MojoSort™ Buffer up to 4 mL and centrifuge the cells at 300 x g for 5 minutes.

Step 7.

Resuspend the cells in 3 mL of MojoSort™ Buffer.
Optional: Take an aliquot before placing the tube in the magnet to monitor purity and yield

Step 8.

Place the tube in the magnet for 5 minutes

DURATION
00:05:00

Step 9.

Pour out the liquid. Resuspend the labeled cells in appropriate amount of buffer.

Step 10.

Repeat steps 7 – 9 on the labeled fraction 2 more times, for a total of 3 magnetic separations.

Optional: Take a small aliquot to monitor purity and yield. If desired, pool the unlabeled fractions and process simultaneously with the positive labeled cells when assessing purity and yield.