MojoSort™ Human CD4 Nanobeads No Wash Protocol

Kelsey Miller

Abstract

This protocol has been optimized to remove washing steps after antibody cocktail and nanobeads incubations, 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 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. To maximize the purity of CD4+ T cells we recommend the depletion of monocytes, as human monocytes express CD4. To deplete monocytes from human PBMCs, we recommend the use of MojoSort™ Human CD14 Selection Kit (Cat. No. 480025/480026). Alternatively, you may consider the Human CD4 T Cell Selection Kit (Cat. No. 480038). After monocyte depletion proceed to isolate the CD4+ T cells. You can also use other methods to deplete monocytes. If you wish to include monocytes in your CD4+ cell isolation, depletion is not required

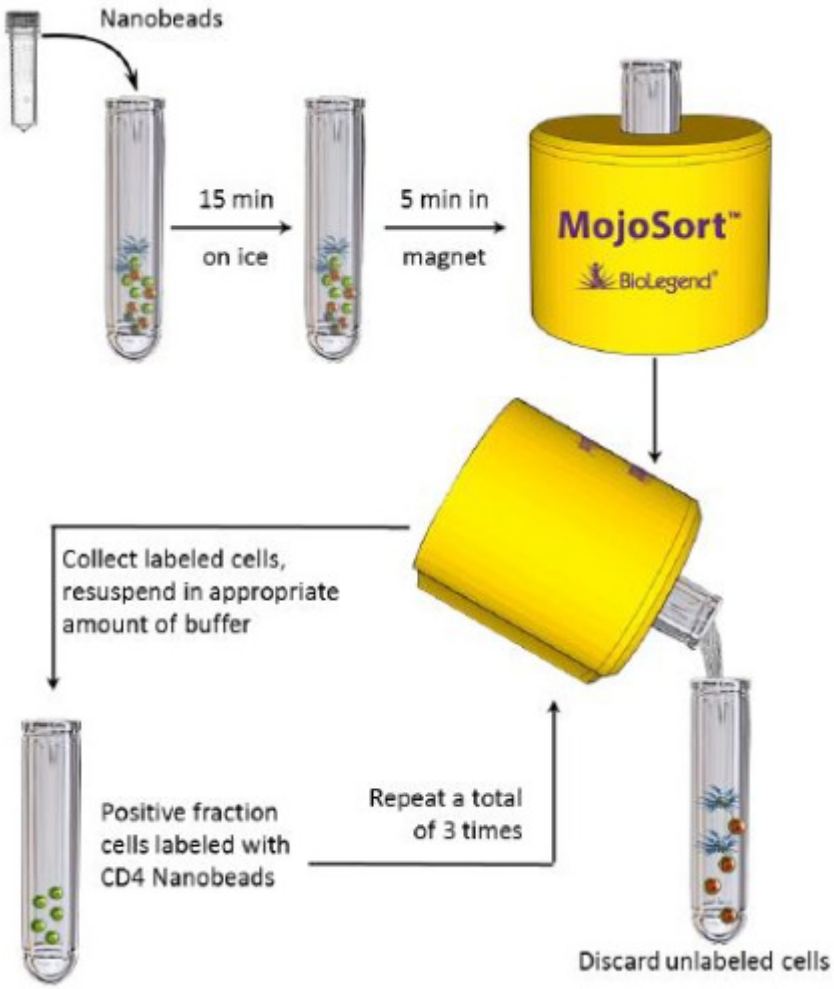
Citation: Kelsey Miller MojoSort™ Human CD4 Nanobeads No Wash Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.e3ibgke
Published: 06 Jun 2016

Guidelines

Product description and procedure summary:
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.

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 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.

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.

DURATION

00:05:00

Step 7.

Place the tube in the magnet for 5 minutes

DURATION

00:05:00

Step 8.

Pour out the liquid. Resuspend labeled cells in appropriate buffer

Step 9.

Repeat steps 6 – 8 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.