MojoSort™ Human CD14 Nanobeads Protocol V.2

DOI
dx.doi.org/10.17504/protocols.io.7×4hpqw

Sam Li[1]

[1]BioLegend

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

Sam Li
BioLegend

DOI: https://dx.doi.org/10.17504/protocols.io.7x4hpqw

External link: https://www.biolegend.com/protocols/mojosort-human-cd14-nanobeads-protocol/4685/

Protocol Citation: Sam Li . MojoSort™ Human CD14 Nanobeads Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.7x4hpqw

Created: October 04, 2019

Last Modified: October 04, 2019

Protocol Integer ID: 28380

Keywords: mojosort, cd14, nanobeads, selection, isolation, cells per tube, targeted cell, specific cell number, nanobead, cell, functional assay, assay

Abstract

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.

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 14 mL 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™ Human CD14 Nanobeads **BioLegend Catalog #**480093

MojoSort™ Buffer **BioLegend Catalog #**480017

- 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

Troubleshooting

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 100 µL of cell suspension (10^7 cells) into a new tube. **Add 5µL of Human TruStain FcX™ (Fc Receptor Blocking Solution)**, mix well and **incubate at room temperature for 10 minutes**. Scale up the volume accordingly if separating more cells. For example, if the volume of Human TruStain FcX™ for $1{\times}10^7$ cells is 5µL, add 50µL for 1×10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

5 Resuspend the beads by vortexing, maximum speed, 5 touches. Add **10µL of Antibody Nanobeads**. Mix well and **incubate on ice for 15 minutes.** Scale up the volume accordingly if separating more cells. For example, add 100 µL of Nanobeads for separating 1×10^8 cells in 1 ml of MojoSort™ Buffer. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

6 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes.

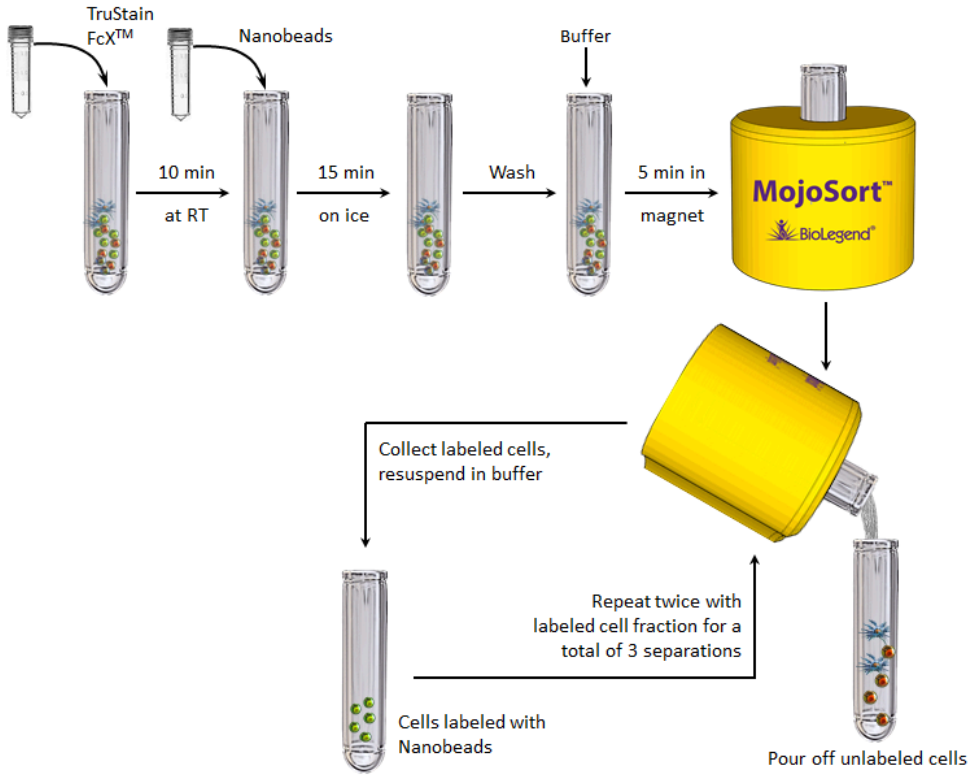
7 Discard the supernatant.

8 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.

9 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.

10 Pour out the unlabeled fraction. If these are your cells of interest, **DO NOT DISCARD**. Resuspend the labeled cells in 2.5 mL MojoSort™ Buffer.

11 Repeat steps 8-10 on the labeled fraction twice more for a total of **3 separations**. Pool the unlabeled fractions and keep the labeled cells. The fraction that is not of interest may be useful as staining controls, to monitor purity/yield, or other purposes.
Optional: Take a small aliquot to monitor purity and yield.


TruStain FcX™
Nanobeads
Buffer
10 min
at RT
15 min
on ice
Wash
5 min in
magnet
MojoSort™
BioLegend
Collect labeled cells, resuspend in buffer
Repeat twice with labeled cell fraction for a total of 3 separations
Cells labeled with Nanobeads
Pour off unlabeled cells