Mojosort™ Mouse Neutrophil Isolation Kit Protocol

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Abstract

Non Neutrophil cells are depleted by incubating the sample with the biotin antibody cocktail followed by incubation with magnetic Streptavidin Nanobeads. The magnetically labeled fraction is retained by the use of a magnetic separator. The untouched cells are collected. These are the cells of interest; do not discard the liquid. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Citation: Kelsey Miller Mojosort™ Mouse Neutrophil Isolation Kit Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.fkabkse
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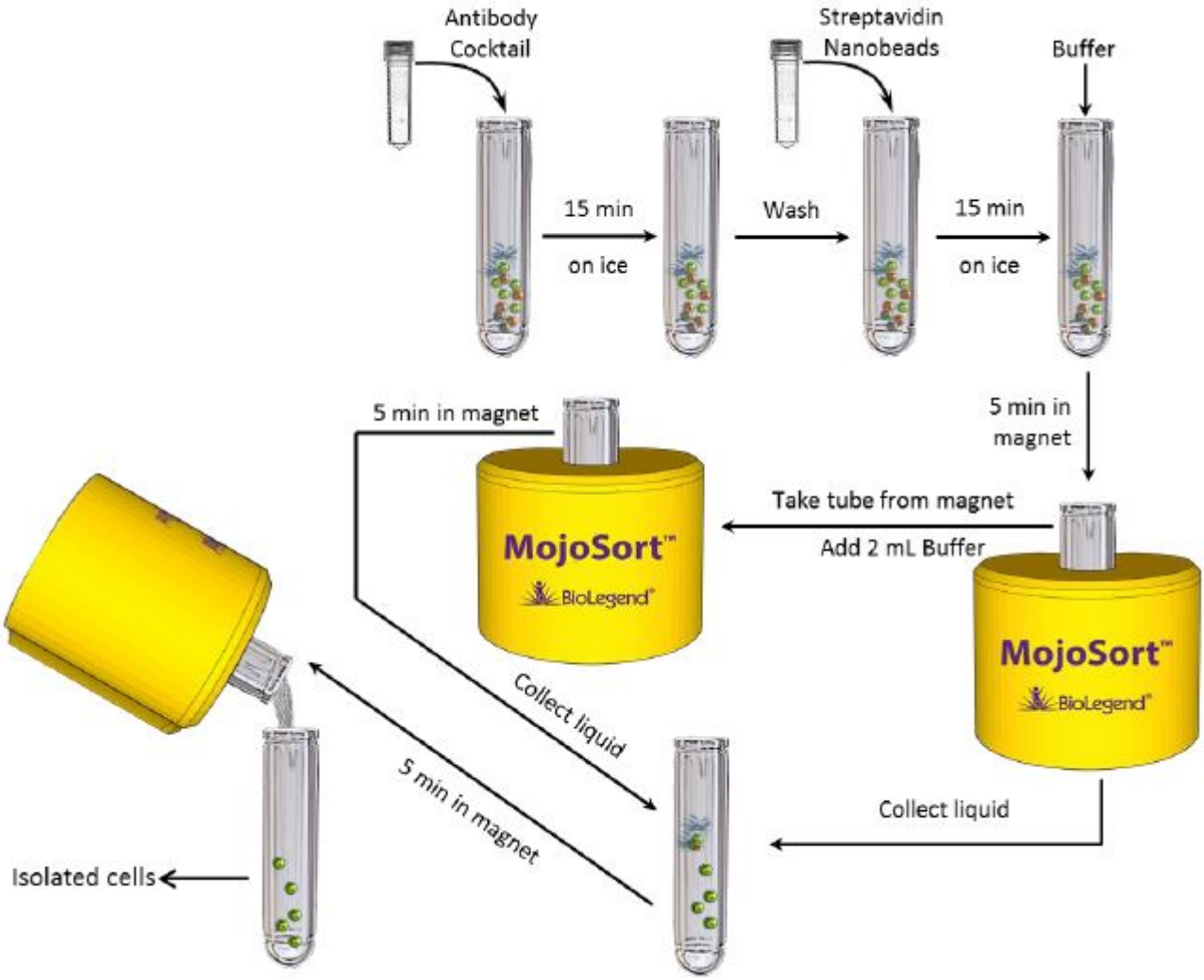
Guidelines

This procedure is optimized for the isolation of 10^7 to 1 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.

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. Please contact BioLegend Technical Service (tech@biolegend.com) for further assistance on how to use MojoSort™ Nanobeads in magnetic separation columns.

Chart Protocol:



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 filling 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.

Step 4.

Aliquot 100 µL of cell suspension (10^7 cells) into a new tube. Add 10 µL of the Biotin-Antibody Cocktail, 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.

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

Step 5.
Wash the cells by adding MojoSort™ Buffer up to 4 mL; centrifuge the cells at 300 x g for 5 minutes.

Step 6.

Discard supernatant and resuspend in 100 µL of MojoSort™ Buffer, or volume needed to keep the cells at 1 x 10^8 cells/mL.

Step 7.

Resuspend the beads by vortexing, maximum speed, 5 touches. Add 10 µL of Streptavidin 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.

Step 8.

Add MojoSort™ Buffer up to 2 mL and place the tube in the magnet for 5 minutes place the tube directly in the magnet for 5 minutes. Collect the liquid in a clean tube. **DO NOT DISCARD.**

Optional: Take an aliquot before placing the tube in the magnet to monitor purity and yield.

Step 9.

Remove the tube from the magnet and resuspend the cells in 2 mL of MojoSort™ Buffer. Place it back into the magnet for 5 minutes. Collect the liquid in the same tube as in step 8. **DO NOT DISCARD.**

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

Step 10.

Take the pooled negative fraction tube (should contain 4 mL in addition to the sample volume and Nanobeads volume) and place the tube in the magnet for 5 minutes.

Step 11.
Pour out and collect the liquid. These are the cells of interest; DO NOT DISCARD.