MojoSort™ Mouse NK Cell Isolation Kit Protocol

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Abstract

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. The purity of the CD3- CD49b+ NK Cell fraction typically ranges from 80 to 90%. Removal of dead cells is highly recommended prior to cell isolation. Dead cells can be removed using appropriate centrifugation media or other methods.

Product description and procedure summary:

The kit is designed for the isolation of mouse NK cells from lymphoid tissues. Target 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 NK Cell Isolation Kit Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.fxpbpmn
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Guidelines

Reagents and instruments required:

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

MojoSort™ Magnet (Cat. No. 480019, 480020) 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 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 10^8 cells/mL.

DURATION
00:05:00

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 volumesfor 10^7 cells.

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

DURATION
00:15:00

Step 5.

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 volumesfor 10^7 cells.

DURATION
00:15:00

Step 6.

Wash the cells by adding 3 mL of MojoSort™ Buffer; centrifuge at 300 x g for 5 minutes, discardsupernatant.

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

DURATION
00:05:00

Step 7.

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 8.
Place the tube in the magnet for 5 minutes.

DURATION
00:05:00

Step 9.

Pour out and collect the liquid. These are your cells of interest;

DO NOT DISCARD.

Step 10.

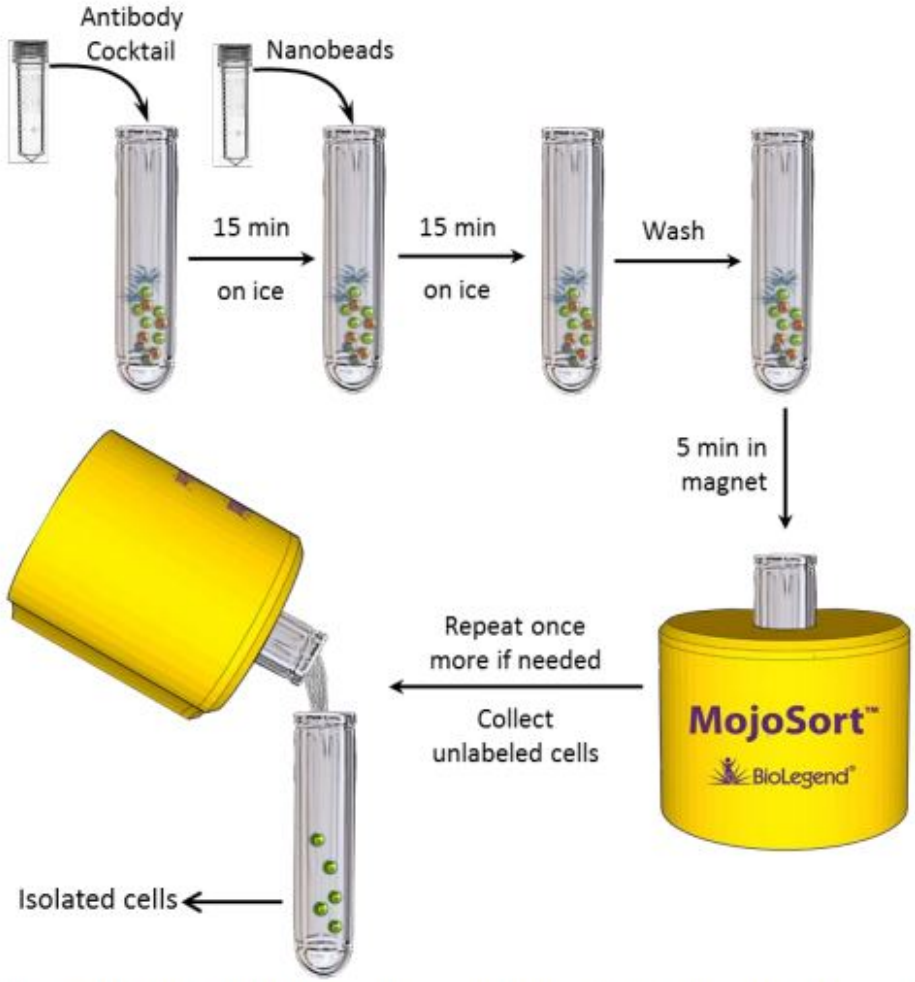
If needed, add 3 mL of MojoSort™ Buffer and repeat steps 8 and 9 with the magnetically labeled fraction up to two times, and then pool the unlabeled fractions.

Note: Repeating the magnetic separation increases the yield, without a strong impact on the purity. The yield will typically increase about 8 – 10% with a second separation, and about 2 – 5% with a third separation. The purity may decrease 1 – 2% with each separation.

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

Step 11.

Chart Protocol:



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.