Oct 04, 2019

MojoSort™ Mouse NK Cell Isolation Kit Protocol

Sam Li[1]

[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.7yshpwe

BioLegend

Sam Li
BioLegend

ABSTRACT

Product description and procedure summary: Target cells are depleted by incubating your sample with the biotin antibody cocktail followed by incubation with magnetic Streptavidin Nanobeads (Cat. No. 480015/480016). 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.

Note: 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. Scale up volumes if using 14mL tubes and Magnet, and place the tube in the magnet for 10 minutes.

Sample Preparation: Dead cell removal prior to cell isolation is recommended to achieve the highest purity and yield of mouse NK cells. Dead cells can be removed using appropriate centrifugation media or by other methods.

EXTERNAL LINK

https://www.biolegend.com/protocols/mojosort-mouse-nk-cell-isolation-kit-protocol/4757/

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

NAME	CATALOG #	VENDOR
MojoSort™ Buffer	480017	BioLegend
MojoSort™ Magnet	480019	BioLegend
MojoSort™ Mouse NK Cell Isolation Kit	480049, 480050	BioLegend

MATERIALS TEXT

- Adjustable pipettes
- 70µm filters (one per sample)
- 5mL (12 x 75mm) or 14mL (17 x 100mm) polypropylene tubes
- Reagents for sample preparation
- Reagents and instruments (Flow cytometer) to determine yield and purity

1 Prepare cells from your tissue of interest or blood without lysing erythrocytes.

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) polypropylene tube.
Note: Keep MojoSort™ Buffer on ice throughout the procedure.

3 Filter the cells with a 70µm cell strainer, centrifuge at 300xg 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.

4 Aliquot 100µL of cell suspension (10^7 cells) into a new tube. Add 10µL 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 of Antibody for separating 1 x 10^8 cells in 1 ml of MojoSort™ Buffer. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.
Optional: Take an aliquot before adding the cocktail to monitor purity and yield.

5 Resuspend the beads by vortexing, maximum speed, 5 touches. Add 10µL of Streptavidin Nanobeads. Mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells. For example, add 100µL of Nanobeads for separating 1 x 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 4mL. Centrifuge the cells at 300xg for 5 minutes.

7 Discard 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 and collect the liquid. These are your cells of interest; **DO NOT DISCARD**. Resuspend the labeled cells in 2.5mL MojoSort™ Buffer.

11 Repeat steps 8-10 on the labeled fraction once more for a total of **2 separations**. Pool the unlabeled fractions. The labeled cells may be useful as staining controls, to monitor purity/yield, or other purposes.
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. The purity may decrease 1-2% with each separation.