# MojoSort™ Mouse Neutrophil Isolation Kit Protocol V.2

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## 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 $2 \times 10^8$ 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.

## 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™ Buffer **BioLegend Catalog #**480017

MojoSort™ Mouse Neutrophil Isolation Kit **BioLegend Catalog #**480057, 480058

- 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

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 × 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 $1 \times 10^8$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 $1 \times 10^8$ 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 Wash the cells by adding MojoSort™ Buffer up to 4mL. Centrifuge the cells at 300xg for 5 minutes.

6 Discard supernatant and resuspend in 100µL of MojoSort™ Buffer.

7 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 100 µL of Nanobeads for separating $1 \times 10^8$ cells in 1 ml of MojoSort™ Buffer. When working with less than $10^7$ cells, use indicated volumes for $10^7$ cells.

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

9 Discard supernatant.

10 Add 2mL 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.

11 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.*

12 Pour out the unlabeled fraction, **DO NOT DISCARD**. Resuspend the **labeled**cells in 2mL MojoSort™ Buffer.

13 Place the tube in the magnet for 5 minutes.

14 Pour out the unlabeled fraction and pool with the previously collected unlabeled cells (should contain ~4mL buffer and cells).

15 Place the pooled **unlabeled** fraction in the magnet for 5 minutes.

16 Pour out the unlabeled fraction, these are the cells of interest, **DO NOT DISCARD**. The labeled fraction may be useful as staining controls, to monitor purity/yield, or other purposes.
*Optional: Take a small aliquot to monitor purity and yield.*