



MojoSort™ Selection Kits Protocol - 5

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Abstract

Product description and procedure summary:

Target cells are either selected or depleted by incubating the 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. If these are the cells of interest; do not discard the liquid. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Sample Preparation:

Enzymatic digestion of mouse brain followed by myelin removal is recommended to achieve the highest purity and yield. There are several protocols published that can be applied. As a general guideline, Trypsin digestion followed by a 70/37/30% percoll gradient will increase final purity and yield. *Scale up volumes if using 14 ml tubes and Magnet, and place the tube in the magnet for 10 minutes.*

Guidelines

Important Note: 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™ Buffer **BioLegend Catalog #480017**

 MojoSort™ Magnet **BioLegend Catalog #480019**

 MojoSort™ Mouse CX3CR1 Selection Kit **BioLegend Catalog #480055, 480056**

 MojoSort™ Mouse P2RY12 Selection Kit **BioLegend Catalog #480113, 480114**

- 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.
- 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 1×10^7 cells/mL.
- 4 Aliquot 100µL of cell suspension (10^6 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 Cocktail for separating 1×10^7 cells in 1 ml of MojoSort™ Buffer. When working with less than 10^6 cells, use indicated volumes for 10^6 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 1×10^7 cells in 1 ml of MojoSort™ Buffer. When working with less than 10^6 cells, use indicated volumes for 10^6 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 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.
- 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. If these are your cells of interest, **DO NOT DISCARD**. Resuspend the labeled cells in 2.5mL MojoSort™ Buffer.
- 13 Repeat steps 10-12 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.

