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MojoSort™ Mouse CD11c Nanobeads Protocol

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

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ABSTRACT

Product description and procedure summary: The cells targeted by the Nanobeads are either selected or depleted by incubating your sample with the directly conjugated magnetic particles. The magnetically labeled fraction is retained by the use of a magnetic separator. After collection of the targeted cells, 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: Enzymatic digestion of mouse spleen is recommended to achieve the highest purity and yield of $CD11c^+$ cells. There are several protocols published that can be applied. As a general guideline, cut mouse spleen into pieces and incubate in 0.5 mg/ml Collagenase for 30 to 60 minutes at room temperature or 37°C. Place the tube in a rocking platform with continuous agitation or gently pipette every 10 minutes. Alternatively, inject 1 ml of enzymes solution in the uncut organ. Force the tissue through a 70µm filter to prepare a single cell suspension, and wash with complete media. Resuspend cells in 0.1 mg/ml DNase 1 solution and incubate at room temperature for 10 minutes. Again, filter cells through a 70 µm filter and wash with complete media. Resuspend in complete media or MojoSort™ Buffer and keep on ice until ready to use.

EXTERNAL LINK

https://www.biolegend.com/protocols/mojosort-mouse-cd11c-nanobeads-protocol/4401/

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 CD11c Nanobeads	480077	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 TruStain FcX (anti-mouse CD16/32 antibody)**, mix well and **incubate at room temperature for 10 minutes**. Scale up the volume accordingly if separating more cells. For example, if the volume of Human TruStain FcX™ for 1x10^7 cells is 10µL, add 100µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

5 Resuspend the beads by vortexing, maximum speed, 5 touches. Add **10µL of Antibody Nanobeads**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume accordingly if separating more cells.

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

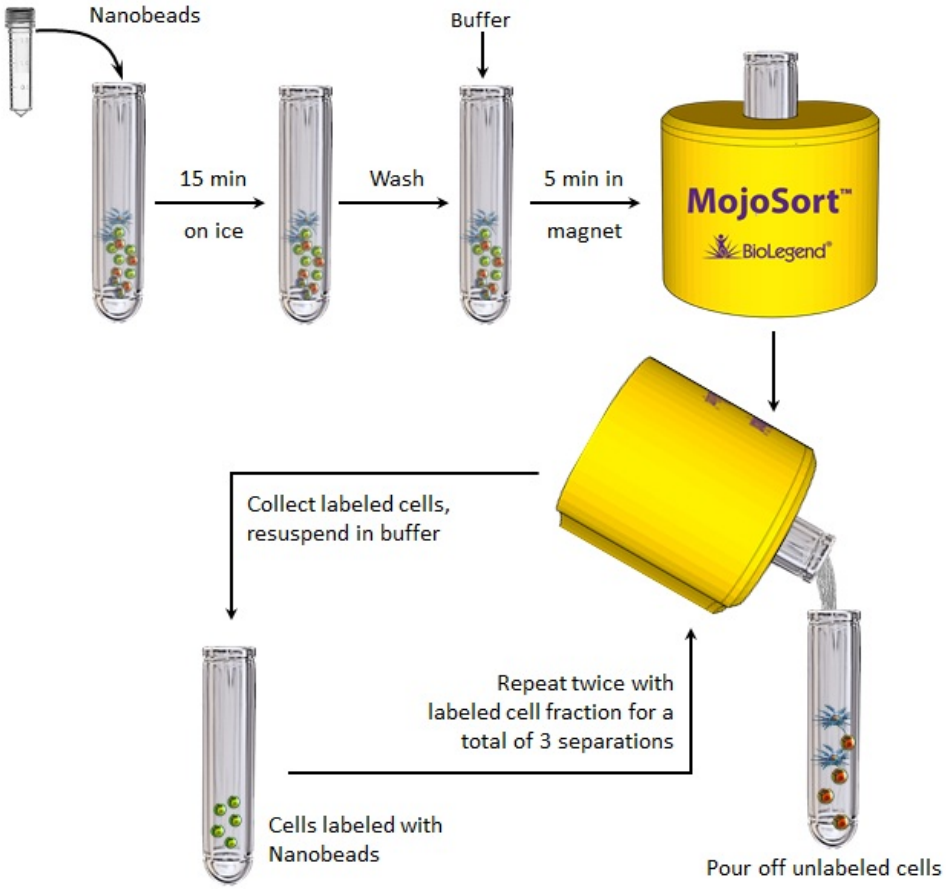
7 Discard the supernatant.

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 the unlabeled fraction. If 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 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.



Assessing Purity and Yield:

12 Stain labeled and unlabeled fractions with PE anti-mouse CD11c (clone N418, cat# 117308) and APC anti-mouse I-A/I-E (clone M5/114.15.2, cat# 107614).
Note: Some CD11c clones may be blocked by the MojoSort™ Mouse CD11c Nanobeads. We recommend adding 0.03 - 0.06 µg of PE anti-mouse CD11c immediately after adding MojoSort™ CD11c Nanobeads (step 5).

13 Acquire the sample and analyze using a flow cytometer and analysis software.

14 The purity of the isolated cells is the percent of live target cells in the labeled fraction.

15 The yield of the isolated cells can be calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Target cell number in labeled fraction}}{\text{Target cell number in labeled fraction + target cell number in unlabeled}} \times 100$$

Alternatively, a cell count can be performed under the microscope using a cell viability solution such as Trypan Blue.