MojoSort™ Mouse CD326 (Ep-CAM) Positive Selection Protocol

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Abstract

Mouse CD326 (Ep-CAM) cells are selected by incubating the sample with biotin conjugated anti-mouse CD326 antibody after blocking with TruStain FcX™ (anti-mouse CD16/32 antibody) then followed by Streptavidin Nanobeads (Cat. No. 480015/480016). The magnetically labeled fraction is retained by the use of a magnetic separator. After collection of the CD326 (Ep-CAM) expressing cells, downstream applications include functional assays, gene expression, phenotypic characterization, etc. In addition to CD326 (Ep-CAM) expression on epithelial and tumor cells, CD326 is also expressed on subtypes of leukocytes, which should be depleted using MojoSort™ Mouse CD45 Nanobeads (Cat. No. 480027/480028) before CD326 positive selection.

Note: This procedure is optimized for the isolation of 10^7 to 2 × 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 CD326 (Ep-CAM) Selection Kit **BioLegend Catalog #**480141, 480142

- 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. Kits for human samples have been optimized for PBMCs, please prepare the cells using a suitable method.

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 × 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 × 10^8 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 Mouse TruStain FcX™ for 1×10^7 cells is 10µL, add 100µL for 1 × 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

5 Add **5µL of the Biotin anti-mouse CD326 (Ep-CAM) antibody**. 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 × 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.

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

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

8 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 × 10^8 cells in 1 ml of MojoSort™ Buffer. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

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

10 Discard supernatant.

11 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.

12 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.

13 Pour out the unlabeled fraction. If these are your cells of interest, **DO NOT DISCARD**. Resuspend the labeled cells in 2.5mL MojoSort™ Buffer.

14 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.