Oct 01, 2019

MojoSort™ Mouse CD326 (Ep-CAM) Selection Kits Column Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.7u4hnyw

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ABSTRACT

BioLegend MojoSort™ nanobeads work in commonly used separation columns, based on our internal research as well as validation by external testing by academic labs. This simple protocol consists of following the MojoSort™ protocol to label the cells with **pre-diluted** MojoSort™ reagents and using the columns as indicated by the manufacturer.

Note: Due to the properties of our beads, it may be possible to use far fewer beads and less antibody than with other commercial suppliers. We recommend a titration to find the best dilution factor. However, as a general rule, dilutions ranging from 1:3 to 1:20 for the Nanobeads can be used. Please contact BioLegend Technical Service (tech@biolegend.com) if further assistance is needed.

EXTERNAL LINK

https://www.biolegend.com/en-us/protocols/mojosort-mouse-cd326-ep-cam-selection-kits-column-protocol

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™.

MATERIALS

NAME	CATALOG #	VENDOR
MojoSort™ Buffer	480017	BioLegend
MojoSort™ Mouse CD326 (Ep-CAM) Selection Kit	480141, 480142	BioLegend

MATERIALS TEXT

Additional reagents:
-commercially available cell separation columns
-5 mL polypropylene tubes
-70 µm cell strainer

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 4mL 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 (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 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 Add **5µL of the pre-diluted Biotin anti-mouse CD326 (Ep-CAM)** antibody. Mix well and **incubate on ice for 15 minutes**. Scale up the volume if separating more cells. For example, add 100µL of pre-diluted Antibody for separating 1 x 10^8 cells in 1ml 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 the supernatant and resuspend cells in 100uL of MojoSort™ Buffer.

8 Vortex the Streptavidin conjugated Nanobeads (to resuspend) at max speed, 5 touches, and prepare the dilutions to test. **Add 10µL of pre-diluted 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 pre-diluted 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.

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

10 Discard the supernatant.

11 Add the appropriate amount of MojoSort™ Buffer and proceed to separation. At least 500µL is needed for column separation.

Note: There are several types of commercially available columns, depending on your application. Choose the one that fits best your experiment:

	Max. number of labeled cells	Max. number of total cells	Cell suspension volume	Column rinse volume	Cell wash volume	Elution volume
Small Capacity	1 x 10^7	2 x 10^8	500µL for up to 10^8 cells	1ml	1 ml	1 ml
Medium Capacity	1 x 10^8	2 x 10^9	500µL for up to 10^9 cells	3ml	3 ml	5 ml
Large Capacity	1 x 10^9	2 x 10^{10}	500µL for up to 10^{10} cells	20-50ml	30 ml	20 ml

Example of magnetic separation with medium capacity columns:

12 Place the column in a magnetic separator that fits the column.

13 Rinse the column with 3 mL of cell separation buffer.

14 Add the labeled cell suspension to the column through a 30 µm filter and collect the fraction containing the unlabeled cells.

15 Wash the cells in the column **3 times** with 3 mL of buffer and collect the fraction containing the unlabeled cells. Combine with the collected fraction from step 3. These cells may be useful as controls, to monitor purity/yield, or other purposes.

16 Take away the column from the magnet and place it on a tube. Then add 5 mL of buffer and flush out the magnetically labeled fraction with a plunger or supplied device. These are the positively isolated cells of interest; do not discard. To increase the purity of the magnetically labeled fraction repeat the isolation process with a new, freshly prepared column.