



## MojoSort™ Selection Kits Column Protocol - 3

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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.

## 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

### MATERIALS

⊗ MojoSort™ Buffer **BioLegend Catalog #480017**

⊗ MojoSort™ Mouse CD3 Selection Kit **BioLegend Catalog #480099, 480100**

Additional reagents:

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

## Troubleshooting



- 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 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 70 µm 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 100 µL ( $10^7$  cells) into a new tube. Add **10 µL of the pre-diluted Biotin-Antibody Cocktail**. Mix well and **incubate on ice for 15 minutes**. Scale up the volume if separating more cells. For example, add 100 µL of pre-diluted 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.
- 5 Vortex the Streptavidin conjugated Nanobeads (to resuspend) at max speed, 5 touches, and prepare the dilutions to test. Add **10 µL 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  $1 \times 10^8$  cells in 1 ml of MojoSort™ Buffer. When working with less than  $10^7$  cells, use indicated volumes for  $10^7$  cells.
- 6 Wash the cells by adding MojoSort™ Buffer up to 4 mL. Centrifuge the cells at 300xg for 5 minutes.
- 7 Discard the supernatant.
- 8 Add the appropriate amount of MojoSort™ Buffer and proceed to separation. At least 500 µL 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	$1 \times 10^7$	$2 \times 10^8$	500 $\mu$ L for up to $10^8$ cells	1ml	1 ml	1 ml
Medium Capacity	$1 \times 10^8$	$2 \times 10^9$	500 $\mu$ L for up to $10^9$ cells	3ml	3 ml	5 ml
Large Capacity	$1 \times 10^9$	$2 \times 10^{10}$	500 $\mu$ L for up to $10^{10}$ cells	20-50ml	30 ml	20 ml

### Example of magnetic separation with medium capacity columns:

- 9 Place the column in a magnetic separator that fits the column.
- 10 Rinse the column with 3 mL of cell separation buffer.
- 11 Add the labeled cell suspension to the column through a 30  $\mu$ m filter and collect the fraction containing the unlabeled cells.
- 12 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.
- 13 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.