MojoSort™ Whole Blood Human CD8 Nanobeads Whole Blood Column Protocol V.2

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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 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™.

- 1 Collect whole blood in collection tube that has anticoagulant, preferably EDTA. Note: Keep MojoSort™ Buffer on ice throughout the procedure.

- 2 Add 1mL of whole blood into a new tube.
- 3 Resuspend the beads by vortexing, maximum speed, 5 touches. Add 50 μ L of pre-diluted conjugated Nanobeads. Mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating larger starting volume of whole blood. For example, add 100 μ L of pre-diluted Nanobeads for separating 2mL of whole blood.
- 4 Proceed to separation on column as indicated by the manufacturer. Note: There are several types of commercially available columns, depending on your application. Choose the one that fits best your experiment.

	Max volum e of whole blood	Colu mn rinse volum e	Cell wash volum e	Elutio n volum e
Who le Bloo d Colu mn	3 mL	3 mL	3x3 mL	5 mL

Example of magnetic separation with medium capacity colu..

- 5 Place the column in a magnetic separator that fits the column.
- 6 Rinse the column with 3mL of cell separation buffer.
- 7 Add the labeled whole blood to the column through a 30 μ m filter and collect the fraction containing the unlabeled cells.
- 8 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.

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- 9 Take away the column from the magnet and place it on a tube. Then add 5 mL of elution 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.