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 that 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™.

Troubleshooting

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 µL 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 µL 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 volume of whole blood	Column rinse volume	Cell wash volume	Elution volume
	Whole Blood Column	3 mL	3 mL	3×3 mL	5 mL

Example of magnetic separation with medium capacity columns:

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 µm 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.

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.