MojoSort™ Positive Selection Columns Protocol

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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.

Citation: Kelsey Miller MojoSort™ Positive Selection Columns Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.e3kbgkw
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Guidelines

Commercially available columns:

	Max. number of labeled cells	Max. number of total cells	Cell suspension volume	Column rinse volume	Cell wash volume	Elution volume
Small Capacity	10^7	$2x10^8$	500 µl for up to 10^8 cells	500 µl	3x1 ml	1 ml
Medium Capacity	10^8	$2x10^9$	500 µl for up to 10^9 cells	3 ml	3x3 ml	5 ml
Large Capacity	10^9	$2x10^{10}$	5 mL for up to 10^9 cells	20 – 50 ml	4x30 ml	20 ml

Example of magnetic separation with medium capacity columns:

a) Place the column in a magnetic separator that fits the column.
b) Wash the column with 3 mL of buffer.
c) Add the labeled cell suspension to the column through a 30 µm filter and collect the fraction containingthe unlabeled cells.
d) Wash the column 3 times with 3 mL of buffer and collect the fraction containing the unlabeled cells.Combine with the collected fraction from step c. These cells may be useful as controls, to

monitorpurity/yield, or other purposes.
e) Take away the column from the magnet and place it on a tube. Then add 5 mL of buffer and flush outthe magnetically labeled fraction with a plunger or supplied device. These are the positively isolatedcells of interest; do not discard. To increase the purity of the magnetically labeled fraction repeat theisolation process with a new, freshly prepared column.

View protocol on Biolegend.com for Data

Protocol

Step 1.
Prepare a single cell suspension and resuspend the cells with ice cold cell separation buffer (MojoSort™ buffer is recommended).

Step 2.

Pass the cells through a 70 µm filter, centrifuge (300 x g for 5 minutes), discard the supernatant and resuspend the cells in cell separation buffer. Adjust the cell concentration to 1 x 10^8 cells/mL.

DURATION
00:05:00

Step 3.

Aliquot 100 µL (10^7 cells) into a new tube.

Step 4.

Vortex the antibody-conjugated Nanobeads (to resuspend) at max speed, 5 touches, and prepare thedilutions to test. Add 10 µL of pre-diluted conjugated 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 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

Note: Depending on the conjugated nanobead you are using, a wash step may be required here.

DURATION
00:15:00

Step 5.

Resuspend the cells in appropriate amount of buffer. 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. See 'Guidelines' for choosing a column for your

experiment.