MojoSort™ Negative Selection Columns Protocol

Kelsey Miller

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 cocktail that with other commercial suppliers. We recommend a titration to find the best dilution factor. However, as a general rule, dilutions ranging from 1:2 to 1:10 for the antibody cocktail can be used. Dilutions ranging from 1:5 to 1:20 for the Streptavidin Nanobeads can be used.

Citation: Kelsey Miller MojoSort™ Negative Selection Columns Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.e3mbgk6
Published: 06 Jun 2016

Guidelines

Commercially available columns:

	Max. number of labeled cells	Max. number of total cells	Cell suspension volume	Column rinse volume	Cell wash/collection volume	Elution volume
Medium Capacity	10^8	$2x10^9$	500 µl for up to 10^8 cells	3 ml	3x3 ml	5 ml
Large Capacity	$2x10^8$	10^9	500 µl for up to 10^8 cells	60ml	30 ml	NA
Extra Large Capacity	10^9	10^{11}	500 µl for up to 10^8 cells	500 ml	200 ml	NA

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. These are the cells of interest; do not discard.
d) Wash the column 3 times with 3 mL of buffer and collect the fraction containing the untouched

cells.Combine with the collected fraction from step c.
e) If desired, the labeled cells can be collected by taking away the column from the magnet and place it ona tube. Then add 5 mL of buffer and flush out the magnetically labeled fraction with a plunger orsupplied device. The labeled cells may be useful as staining controls, to monitor purity/yield, or otherpurposes.

See Biolegend.com for Data

Protocol

Step 1.
Prepare a single cell suspension and resuspend the cells with ice cold cell separation buffer (MojoSort™ buffer 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. Add 10 µL of the pre-diluted Biotin-Antibody Cocktail, mixwell and incubate on ice for 15 minutes. Scale up the volume if separating more cells. For example, add 100 µL of pre-diluted antibody cocktail for separating 1 x 10^8 cells in 1ml of buffer. When working withless than 10^7 cells, use indicated volumes for 10^7 cells.

DURATION
00:15:00

Step 4.
Add cell separation buffer up to 4 mL; centrifuge the cells at 300 x g for 5 minutes.

DURATION
00:05:00

Step 5.
Discard supernatant and resuspend in 100 µL of buffer.

Step 6.

Vortex the Streptavidin conjugated Nanobeads (to resuspend) at max speed, 5 touches, and preparethe 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 x 10^8 cells in 1ml of buffer. When working with less than 10^7 cells,use indicated volumes for 10^7 cells.

Note: Depending on the isolation kit you are using, a wash step may be required here. If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.

DURATION
00:15:00

Step 7.

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.