MojoSort™ nanobead cell isolation using a commercial magnetic column provides high purity, yield, and preserved cell function.

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Abstract

Magnetic cell sorting has been widely used to isolate different cell types as it is a fast and reliable method to obtain discrete populations with high purity, and yield. Several platforms have been developed to isolate the cells. The two more widely used are based on columns and stand alone magnets. As researchers balance the equivalence between these two systems, we evaluate important parameters when using the two methods, and the use of BioLegend reagents in magnetic separation columns.

Introduction

To address possible concerns associated with functionality, cells were isolated with our recently developed MojoSort™ cell separation reagents using commercially available magnetic cell separation columns. The same sample was processed with similar commercial products associated with the magnetic columns. The binding of the magnetic nanoparticles to the isolated cells was imaged by electron microscopy, and cellular functionality was tested by activation of MAPK/ERK pathway and cell proliferation assays. Our data reveals that cells isolated with MojoSort™ reagents using isolation columns only have very few nanoparticles on the cell surface. Furthermore, negatively selected cells using MojoSort™ reagents with either a MojoSort™ separator or magnetic columns do not display nanobeads on the cell surface, and phosphorylation of ERK1/2 level did not increase as detected by western blot. Finally, when analyzing positively selected cells using a MojoSort™ kit with a MojoSort™ magnet, we observed a higher number of nanoparticles on the cell surface as compared to cells isolated using separation columns, but the cells still maintain their functionality (data not shown). Thus, our data demonstrates that cells isolated with MojoSort™ reagents are viable and functional, independently of the magnetic separator used, either column-based or handheld magnet.

Methods

Cell Separation

A single cell suspension from C57BL/6 mouse lymphoid tissues or human peripheral blood mononuclear cells was prepared to isolate indicated cell types with MojoSort™ or commercial magnetic columns and their associated reagents. The isolated cell purity was assessed by using cell type-specific markers for flow cytometric analysis. Dead cells were excluded by 7-AAD.

Electron microscope image

Cells were fixed, embedded, sectioned and stained following a standard protocol and UCSD core facility recommendations. Grids were viewed using a JEOL 1200EX II (JEOL, Peabody, MA) transmission electron microscope.

Western blot

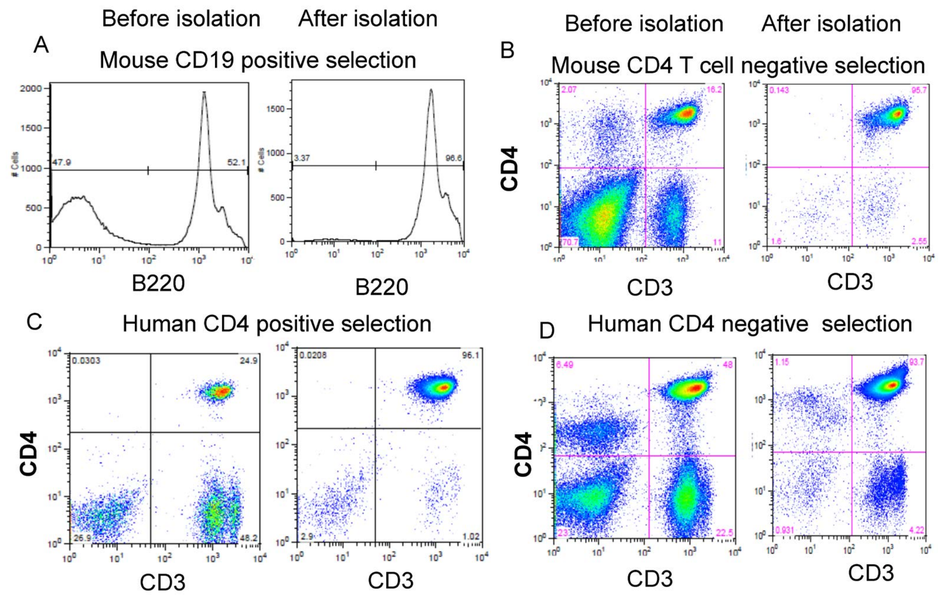
Cells were lysed with RIPA Lysis buffer. Whole cell lysates were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Phospho ERK1/2 (Thr202/Tyr204). Proteins were visualized using a donkey anti-rabbit secondary conjugated to HRP and a chemiluminescence detection system according to BioLegend western blot standard protocol.

Cell proliferation

Isolated $CD4^+$ cells (1x 10^6 cells) were stimulated with plate-bound anti-mouse CD3 antibody and soluble anti-mouse CD28 for 3 days. After incubation, cell proliferation was measured with BioLegend Deep Blue Cell Viability™ Kit, based on resazurin. Resazurin, measures the metabolic activity of living cells. The relative fluorescence intensity was measured after a 7 hour incubation using a SPECTRAmax Gemini XPS fluorescence microplate.

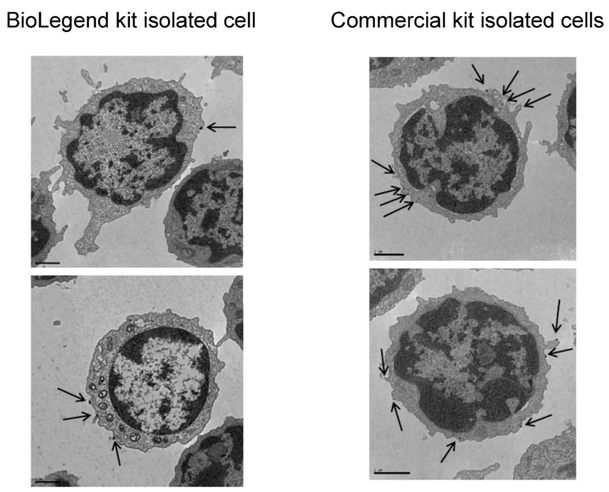
Results

Figure 1. MojoSort™ Nanobeads can be used in a commercial magnetic column for positive and negative selection



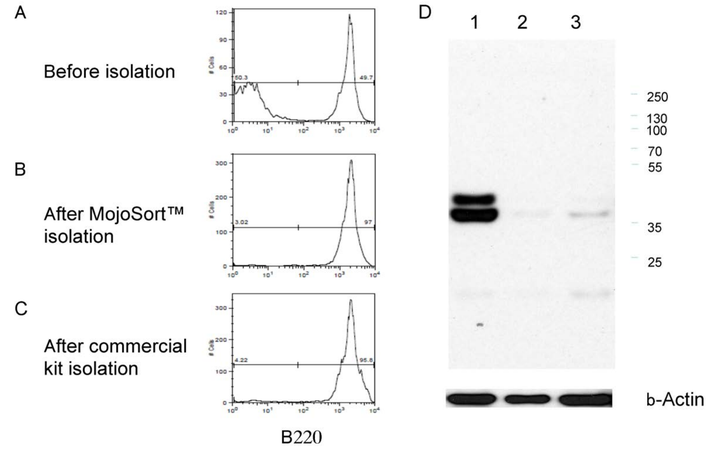
MojoSort™ reagents were used in commercially available columns to isolate A) Mouse $CD19^+$ cells, positive selection with directly conjugated MojoSort™ Nanobeads; B) Mouse $CD4^+$ T cells, negative selection; C) Human $CD4^+$ T cells, positive selection, and D) Human $CD4^+$ T cells, negative selection.

Figure 2. Positively isolated cells with MojoSort™ Nanobeads using columns show low number of magnetic nanoparticles on the cell surface



Positively selected mouse $CD19^+$ cells using BioLegend (left) and another commercial kit (right) were fixed, embedded, sectioned and imaged by electron microscope. Arrows indicate magnetic nanoparticles.

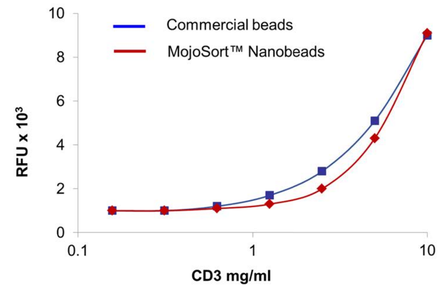
Figure 3. MojoSort™ Nanobead-isolated cells have minimal activation during the isolation process



BioLegend (B and D lane 2) and other commercial (C and D lane 3) mouse CD19 nanobeads isolated cells and PMA stimulated splenocytes (Lane 1) were lysed with RIPA Lysis buffer and probed with anti-Phospho ERK1/2 (Thr202/Tyr204). Protein content was visualized using a donkey anti-rabbit secondary conjugated to HRP and a chemiluminescence detection system according to BioLegend western blot standard protocol.

Figure 4. Functionality is similar between cells isolated with columns, using either MojoSort™ Nanobeads or column associated reagents

Type of nanomagnetic particle used	% PURITY	% YIELD
MojoSort™ CD4 Nanobeads	92.4	65
CD4 beads recommended for the columns used for isolation	91.5	67



C57BL/6 mouse splenocytes were isolated with MojoSort™ Mouse CD4 Nanobeads (red) and a commercial mouse CD4 positive selection kit (blue) using columns. After isolation, CD4 positive cells were cultured with plate-coated CD3 and soluble CD28 for 3 days.

Conclusions

1. MojoSort™ Nanobeads can be used in a commercial magnetic column for positive and negative selection.
2. In commercial columns, positively isolated cells using MojoSort™ Nanobeads have very few cell-bound nanobeads.
3. MojoSort™ Nanobeads isolated cells only have minimal activation during the isolation process.
4. MojoSort™ Nanobeads positively isolated cells maintain cell functionality.

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