RESEARCH SNAPSHOT

Simultaneous Depletion of CD45+ Cells and Enrichment Of Viable Cells With LeviSelect and LeviCell

Overview

For oncology research, tumor tissue is a valuable and often precious sample, and it is critical for therapeutic and diagnostics research. Scientists often use dissociated tumor cells (DTCs) as a viable alternative for fresh tumor cells to study cancer phenotype due to their longer shelf life and accessibility. DTCs are single-cell suspensions generated from fresh primary tumors using enzymatic and mechanical dissociation. It recapitulates the tumor microenvironment and contains all the cell populations present in the tumor microenvironment, such as tumor cells, immune cells, and other support cells. It is often necessary to remove immune cells in tumor samples to characterize the genetic modulations in the cells, especially in treatment-resistant tumors. Immune cells express a unique transmembrane protein, 'CD45', on their cell surface that most tumor cells do not express (exception for blood tumors). CD45 can be targeted and used to separate the CD45+ immune cells from CD45- tumor cells.

The LeviSelect™ Human CD45 Depletion Kit (PN 1004001) is designed to selectively deplete CD45+ cells in fresh or previously frozen human tissue samples with the help of superparamagnetic nanospheres conjugated with anti CD45 antibodies. The samples were incubated with the magnetic nanospheres, which interact with CD45+ cells to form cell: bead complexes. When the samples passed through the LeviCell™ System (PN 1000001), the unwanted CD45+ cells were retained against the bottom and sides of the cartridge due to the magnetic forces on the bound magnetic nanospheres. As Levitation Technology is gentle and primarily used for viable cell enrichment, users achieve simultaneous enrichment of live cells and depletion of CD45+ cells. Live CD45- cells are recovered in their native state and can be used for downstream processing after collection.

HIGHLIGHTS

- Tumor tissues show variable infiltration
- More efficient characterization of tumor cells require depletion of the immune cell fraction
- Simultaneous viable tumor cell enrichment and >99% depletion of immune cells can be achieved with LeviSelect and LeviCell

DTCs from various tumor tissues (Table 1) were selected for analysis to encompass a range of common tumor types. There are multiple methods for measuring the depletion of CD45 positive cells and the efficiency of the LeviSelect Human CD45 kit (such as flow cytometry, single-cell RNA sequencing, quantitative polymerase chain reaction, and immunofluorescence). This report analyzes outputs using flow cytometry with two markers, CD45 (for CD45 positive cells) and Epithelial Cell Adhesion Molecule (EpCAM) for epithelial cells. DTC samples were procured from Discovery Life Sciences, washed with recommended protocol, and then incubated for 5 minutes with anti-human CD45 nanospheres. Levitation agent was added to a final concentration of 150mM and processed in the LeviCell System per the standard protocol. After sample enrichment, cells were collected, labeled with EpCAM-PE-Cy7 and CD45-FITC antibodies and analyzed on the Sony Biotechnology SH800 instrument. Dead cells were excluded by 7AAD. The analysis shows more than 99% depletion of CD45+ cells across all tumor sample types.

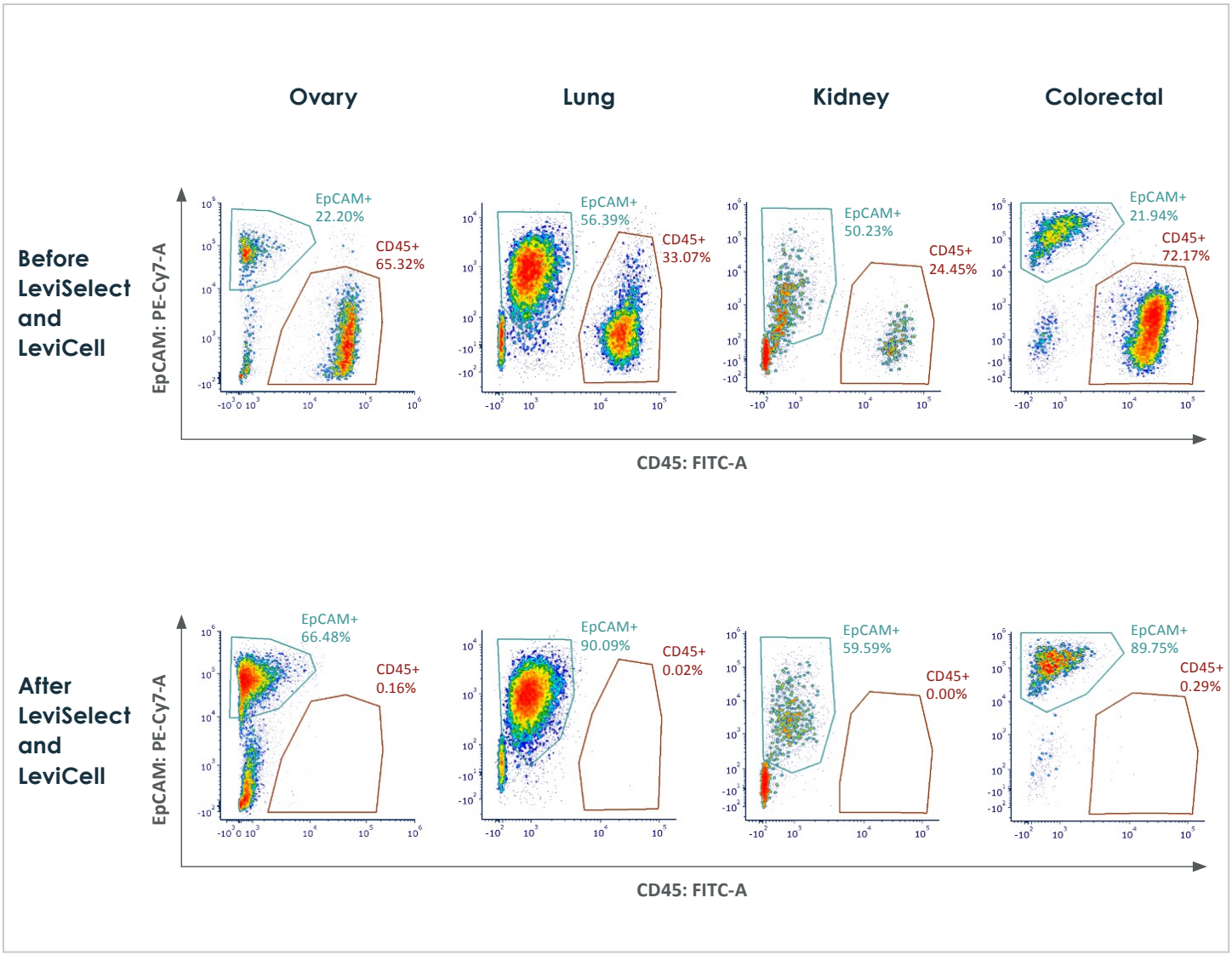

Figure 1. Flow cytometry analysis to measure the depletion of CD45+ cells from diverse dissociated tumor cells. *Cells were first gated on forward scatter (FSC-A) vs. side scatter (SSC-A). Next, FSC-H (height) vs. FSC-A (Area) was used to select single cells. Single cells were then gated for 7AAD (cell death marker) to discard cell debris and dead or dying cells. Live cells were then gated for EpCAM-PE-Cy7 for epithelial cells (Y-axis) and CD45-FITC for CD45+ cells (X-axis). %CD45 depletion was calculated after the LeviCell run. Note that since only two antibodies were used for staining, a percentage of cells are stained double negative.*

$$\text{\% CD45+ Depletion} = 100\% - \frac{\text{Number of viable CD45+ cells after depletion}}{\text{Number of viable CD45+ cells before depletion}}$$

Flow cytometric analysis of lung, ovary, kidney, and colorectal tumor samples demonstrate greater than 99% depletion of CD45+ cells after using the LeviSelect Human CD45 Depletion Kit with the LeviCell System. Additionally, viability was improved in all samples.

DTC sample	Input viability	Output viability	CD45+ cells before depletion	CD45+ cells after depletion	% Depletion of CD45+ cells
Lung (Sarcomatoid Carcinoma)	39%	87%	33.37%	0.03%	99.9%
Ovary (Adenocarcinoma)	43%	81%	65.72%	0.16%	99.9%
Kidney (Oncocytoma)	43%	86%	23.98%	0%	100%
Colorectal (Adenocarcinoma)	49%	85%	71.70%	0.35%	99.6%

Table 1. Simultaneous CD45+ immune cell depletion and tumor cell enrichment across four different tumor samples. *Tumor samples were depleted for CD45+ cells with LeviSelect and LeviCell 1.0. The total Live cell number in the input and output was counted using a Nexcelom Spectrum Cellometer and AO/PI dyes. The number of CD45+ cells before and after depletion was determined by multiplying the live number of cells counted from the cellometer with the % CD45+ from flow cytometry. Each sample was processed in two independent replicates, and the average values are reported here. Output viability may have been affected by the removal of CD45+ cells. In this assay, a large pool of viable CD45+ cells is intentionally depleted and thus may affect the total viable count of the cells. Simultaneous enrichment of viable cells and depletion of immune (CD45+) cells were achieved in a single LeviCell run.*

Conclusion

The LeviSelect Human CD45 Depletion Kit depleted >99% CD45+ cells while simultaneously enriching the viable cell fraction from diverse tumors using the LeviCell System. Therefore, the LeviSelect Human CD45 Depletion Kit can be an effective tool for depleting CD45+ immune cells and enriching viable tumor cells for downstream analysis.

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