TECHNICAL NOTE

Depletion of High Cell Numbers from a Mixed Population Using LeviSelect

Overview

The LeviCell™ platform can separate cells based on physical properties including density and magnetic susceptibility. The level of separation between cells in a mixed cell population is most effective with large differences in cellular density or magnetic susceptibility. One limitation of the technology, however, occurs when a user would like to separate a cell population of interest from an unwanted cell population with a very similar density. In this scenario, both populations levitate to the same equilibration height within the cartridge separation channel.

To improve separation of unwanted cell populations, we are developing methods to alter the density of a specific cell population by binding objects of lower or higher density (than the cells of interest) via ligand binding to a target surface protein. In addition to altering density alone, it is also possible to alter the magnetic properties of an unwanted population by binding magnetic particles to those cells via ligand binding to target surface proteins. Since the LeviCell instrument contains strong fixed magnets, the unwanted population can be depleted from the sample during levitation.

Levitation Technology utilizes a simple workflow, requiring the addition of a levitation agent (e.g. 150 mM, final) and typically 20 minutes of levitation time within the LeviCell, and offers viable cell enrichment for most cells. Levitation itself requires no cell staining, labeling, or use of cell surface antibody markers. The gentle process virtually eliminates undue stress on cells and allows users to ultimately analyze their cells as close to their in vivo state as possible. Combining levitation with magnetic particle separation allows for enrichment of a high viability desired cell population in a single, simple, gentle workflow.

Introduction to Magnetic Bead Depletion

The LeviSelect™ Human CD45 Depletion Kit consists of superparamagnetic nanospheres conjugated with an anti-human CD45 antibody. A 200 µL single cell suspension of cells may be depleted by incubating with 20 µL of the bead suspension for 5 minutes. Afterwards, levitation agent is added to a final concentration of 150 mM and 220 µL of the mixture is loaded onto the LeviCell, without washing. The fixed magnets within the LeviCell will attract the bead-bound cells to the bottom and side walls of the cartridge, while the unbound cells will levitate within the paramagnetic levitation buffer, separating live cells from dead equilibrium heights. After the unbound cells have reached equilibrium, the live, human CD45-depleted cells will be collected in the outlet well and are ready for downstream processing. The total workflow takes ~30 minutes.

Processing Greater Than 1M Total Cells

The cell size and number of cells loaded guides the ease of visibility and differentiation of the live cell band from the dead/dying cell band to set a proper split line value leading to the highest output yield and viability. Above 1M cells, the live and dead cell bands increase in thickness and can becomes more difficult to distinguish (Figure 1). This may affect the final yield and viability of the live cells. When performing depletion on the LeviCell 1.0, a significant portion of the sample is immobilized against the bottom of the separation channel within the first minute of levitation and are not inhibitory to the levitation of the remaining cells. This technical note explores the feasibility and consequences of performing human CD45 depletion on the LeviCell 1.0 system with samples containing more than 1M total cells.

A broad range of input cell numbers were tested, from 0.5M to 8M $CD45^{Pos}$ cells. All depletion samples contained a mixture of human PBMCs ($CD45^{Pos}$) and H358

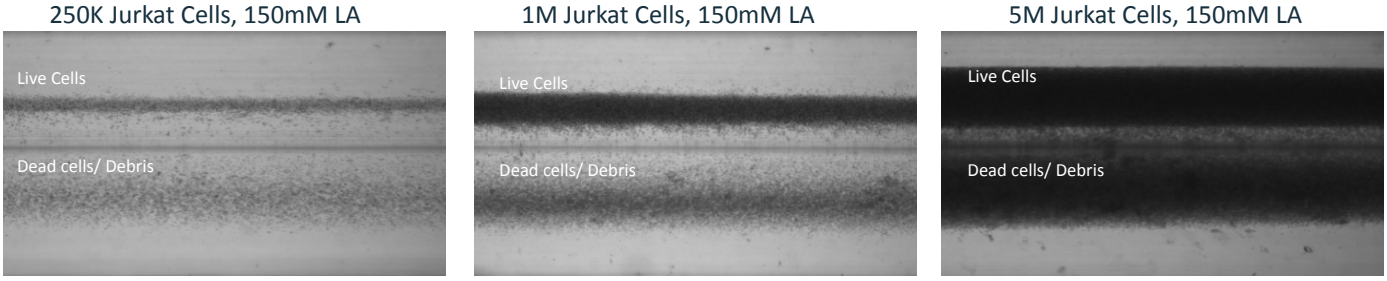

Figure 1: Live cell band visualization is proportional to the number of cells loaded into the LeviCell System. *Different numbers of aged Jurkat cells were loaded (250K, 1 million and 5 million) using 150 mM LA and their levitation height captured at 20 min post levitation. Note the broadening of the live cell band as the number of cells increase. The distance between the live and dead cell bands does not change, but the cell bands expand as more cells are loaded, giving the impression that the separation distance is decreasing.*

Depletion was performed using the LeviSelect Human CD45 Depletion Kit per the standard protocol. $CD45^{Pos}$ and $CD45^{Neg}$ cells were counted with the Nexcelom Cellometer Spectrum using fluorescence detection before and after the LeviCell run. These cell counts were used to calculate depletion, yield, and purity using the below equations:

$$Depletion = \left(1 - \frac{\#\ live\ CD45^{Pos}\ cells\ in\ output}{\#\ live CD45^{Pos}\ cells\ in\ input}\right) * 100\%$$

$$Yield = \frac{\#\ live\ CD45^{Neg}\ cells\ in\ output}{\#\ live CD45^{Neg}\ cells\ in\ input} * 100\%$$

$$Purity = \frac{\#\ live\ CD45^{Neg}\ cells\ in\ output}{total\ \#\ live\ cells\ in\ output} * 100\%$$

In Figure 2, the results are summarized with depletion, yield, and output purity plotted against the total number of $CD45^{Pos}$ cells in the input sample. Each depletion sample is also color coded to the input purity, which ranged from 0.2% to 40% $CD45^{Neg}$. Across all runs compiled here, the average depletion was 99.6% and there was no trend with increasing number of $CD45^{Pos}$ cells. Similarly, the average yield of $CD45^{Neg}$ cells was ~60% and, likewise, did not trend with the number of $CD45^{Pos}$ cells in the input. This demonstrates the standard LeviSelect protocol can deplete up to 8M $CD45^{Pos}$ cells without loss in functionality or loss of desired cells. There is a negative trend in output purity with the number of $CD45^{Pos}$ cells in the input. However, this is expected as the depletion rate is constant. For the samples with 8M $CD45^{Pos}$ cells, after 99.6% are depleted, there are expected to be ~30,000 $CD45^{Pos}$ cells remaining. For samples with low initial purity, such as those with 0.2% and 0.5% $CD45^{Neg}$, this will represent a larger portion of the output fraction than higher input purity samples resulting in lower output purities. This highlights the need to understand the relative proportion of $CD45^{Pos}$ cells in the sample prior to depletion.

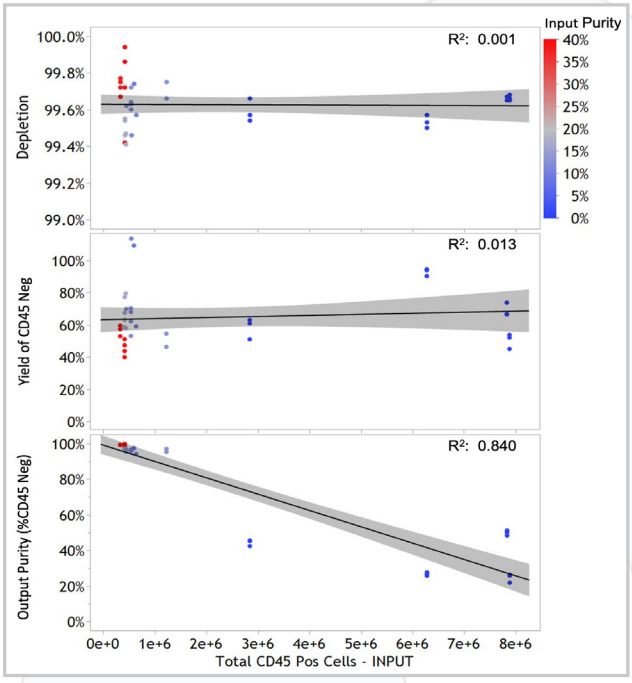

Figure 2: *Depletion of $CD45^{Pos}$ cells, yield of $CD45^{Neg}$ cells, and output purity versus total number of $CD45^{Pos}$ cells in the input. Shadowing around the best fit line indicate a 95% confidence interval.*

Conclusion

The data shown in this technical note demonstrate that samples with high input cell numbers of $CD45^{Pos}$ cells can be processed using the LeviSelect Human CD45 Depletion Kit on the LeviCell System. 1M CD45 negative cells can be enriched independent of total number of cells loaded (max shown at 9M cells). Majority of the $CD45^{Pos}$ cells are removed in the first minute after sample introduction into the separation channel, with most bead-bound cells quickly dropping to the bottom channel. The remaining $CD45^{Neg}$ cells are free to levitate as per normal viable cell enrichment. Therefore, samples with more than 1M total cells can be successfully processed with the LeviSelect Human CD45 Depletion Kit.