Impact of Cell Split Timing on Staining Efficiency in Flow Cytometry Using Daudi and K562 Cell Lines

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Natural killer (NK) cells play a critical role in the immune system by eliminating malignant and infected cells through two primary mechanisms: direct killing and antibody-dependent cellmediated cytotoxicity (ADCC). Both pathways contribute to immune defense and are of interest in the Denton Immunology lab. This lab focuses on studying and optimizing NK cell function by assessing factors that influence these killing mechanisms using Daudi and K562 cancer cell lines. Flow cytometry is a key tool in these investigations, providing insight into NK cell activity and target cell characteristics. However, variations in cell culture conditions, such as the timing of cell splits before an assay, may impact staining quality and data interpretation. To evaluate this, staining data from Daudi and K562 cells that were split either one or two days before flow cytometry will be analyzed. Statistical comparisons will determine whether split timing significantly affects staining intensity and overall data quality. It is hypothesized that cells split two days before the assay will exhibit better staining quality due to more stable surface marker expression and reduced cellular stress. If confirmed, this finding could help refine best practices for cell culture preparation in flow cytometry experiments. These findings could provide guidance on whether cell splits should be standardized at a specific time before assays to ensure optimal staining quality and data consistency. If split timing is shown to have a significant impact, this information could help the lab and others refine experimental workflows, improving reproducibility and reliability in flow cytometry-based studies. The project described was supported in part by an Institutional Development Award (IDeA) from the NIGMS of the National Institutes of Health under Grant # 5P20GM103427.