nanocellect Biomedical, Inc.

Application Note

Sorting T Cell Subsets with the WOLF G2® Cell Sorter

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Introduction

T cells are of great interest to researchers and clinicians because of the critical role they play in the immune system. Recently, T cells have moved from the laboratory to revolutionary therapeutics in oncology, e.g. CAR-T, as well as vaccine development. As a result, T cell subset isolation is critical to researchers as there are many different types of T cells that play different roles within the immune system. However, isolating specific T cell subsets can be challenging since it requires the use of several epitope binding antibodies.

Fluorescence-activated cell sorters are exceptionally appropriate instruments that address this challenge by allowing researchers to label cells with multiple fluorescent antibodies and easily identify different T cell subsets within a cell population. A challenge with traditional droplet-based sorters is that they use high-pressure that can result in a significant amount of shear stress and lead to low viability. Furthermore, the non-disposable fluidics of these traditional sorters can also result in confounding cross-contamination between samples or the presence of microbes that contaminate samples.

The WOLF G2 Cell Sorter is a gentle microfluidic cell sorter equipped with 2 lasers in 3 configurations: 488/405nm, 488/561nm and 488/637nm; and up to 9 fluorescent channels. In addition, the WOLF G2 has the ability to sort 2 target populations at the same time and dispense single cells into 96- or 384-well plates with the N1 Single Cell Dispenser. Most importantly, the G2 sorts cells with less than 2 psi and uses completely disposable fluidics that enable high viability and eliminate cross-contamination or microbial growth. Here we used the G2 with the 488/637nm and 488/405nm laser configurations to identify and sort CD4+ and CD8+ T cells, simultaneously.

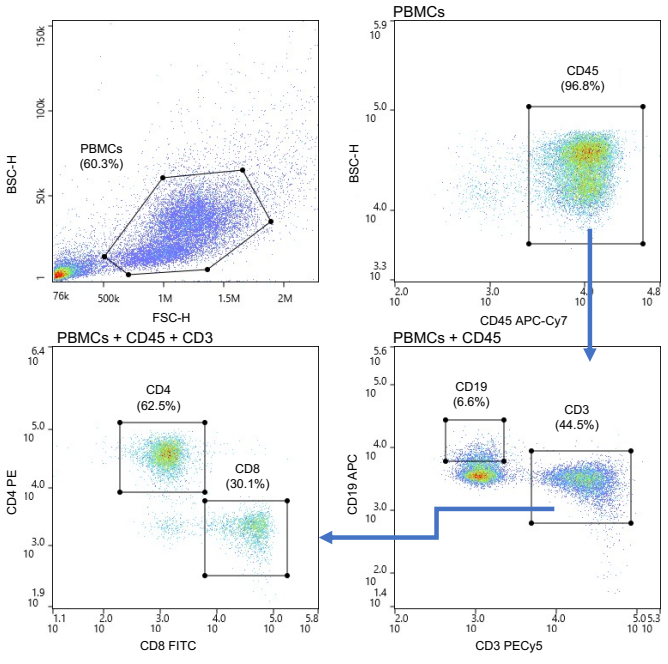

Figure 1. CD4 and CD8 T cell gating strategy: PBMCs were identified via BSC/FSC scatter plot and white blood cells were further identified with CD45. CD3+ and CD19+ cells were then identified through the CD45+ gate and finally CD4+ and CD8+ T cells were gated through the CD3+ gate.

Methods

BioLegend's Veri-Cell™ PBMCs (BioLegend, #425004) were prepared according to the manufacturer instructions and diluted in sample buffer of PBS + 1% BSA. 1 x 106 cells were resuspended in 50 µL of sample buffer and blocked with 5% True-Stain Monocyte Blocker (BioLegend, #426102) for 10 minutes at room temperature. For the 488/637nm laser configuration, cells were then stained with BioLegend antibodies CD45 APC-Cy7 (#30414), CD8 FITC (#301106), CD4 PE (300508), CD3 Pe-Cy5 (#300410), and CD19 APC (#302212). Cells were stained with CD45 Pacific Blue (# 36859), CD4 AF488 (#317419), CD8 BV510 (#300933), CD3 BV570 (#300435) and CD19 PE (#302208) for the 405/488nm configuration. Following a 20-minute incubation, the samples were washed and resuspended to 500,000 cells/mL. Single color compensation controls were also prepared. Compensation was applied to the PBMCs sample within the WOLFViewer software. T cells were identified as CD45+ CD3+ CD19- and CD4+ and CD8+ populations identified by being positive for either CD4 or CD8 (Figure 1). Both CD4+ and CD8+ T cells were sorted at the same time and purity was accessed by re-analyzing the samples on the WOLF G2. This was repeated in triplicate, including the use of new cartridges and tubing sets for each replicate to eliminate potential cross contamination.

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Results

Using the WOLF G2 in the 488/637nm configuration, CD4+ and CD8+ T cells were sorted with an average purity of 93 ± 5.3% and 91± 3.7%, respectively. This is a 3.2-fold enrichment of CD4 T cells and a 5-fold enrichment of CD8 T cells in the PBMC population.

With the WOLF G2 in the 488/405nm configuration, high purities of CD4+ and CD8+ T cells were also observed with an average purity of 94± 2.5% and 94± 2.5% respectively. This resulted a 3.3-fold enrichment of CD4 T cells and an 8.5-fold enrichment of CD8 T cells in the PBMC population.

Conclusion

The WOLF G2 488/637nm laser configuration has up to 7 fluorescent channels and the WOLF G2 488/405nm laser configuration has up to 9 fluorescent channels along with its gentle sorting technology and disposable fluidic system. Here, 5 fluorescent markers were used to confidently identify CD4+ and CD8+ T cells from a PBMC population. Furthermore, sorting both the CD4+ and CD8+ T cells simultaneously resulted in an average purity > 90% for both configurations. This experiment demonstrates the broad capabilities of WOLF G2 and highlights the potential sorting possibilities that are now achievable.

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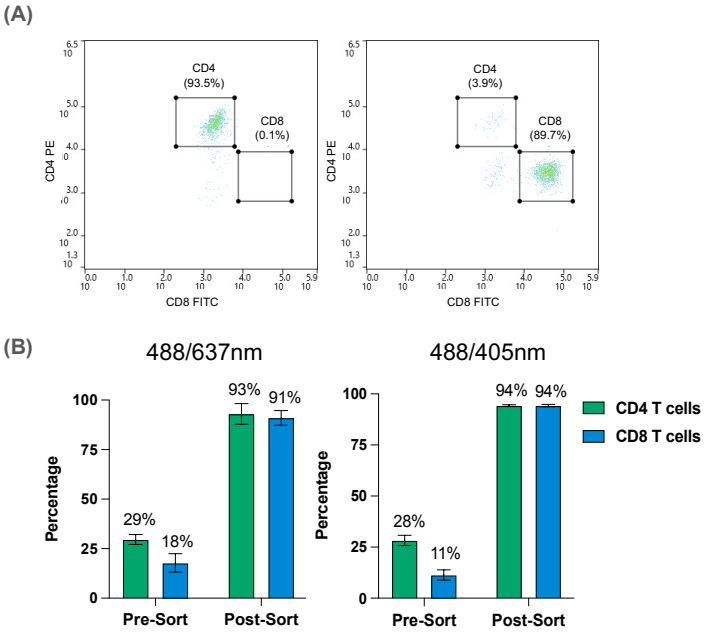

Figure 2. CD4 and CD8 T cell post sort analysis: (A) Representative post-sort purity results **(B)** Average pre-sort and post-sort purity after sorting for CD4 and CD8 T cells concurrently (n=3) for the 488/637nm and 488/405nm laser configuration.

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