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Immunophenotyping On The Cytek® Muse® Micro Cell Analyzer Using Cytek® Reagents

Simplified immunophenotyping is essential for a range of immunology workflows, particularly when evaluating whole blood or peripheral blood mononuclear cell (PBMC) samples to assess the quality of samples before more complex experiments. These analyses provide critical information such as antibody titers, data on immune cell populations (T cells, B cells, and others), and the purity or identity of cell isolations. Simple assays using one to three colors, combined with forward scatter (FSC) and/or side scatter (SSC), can provide both population percentages and absolute counts. Data can be easily obtained by novice and experienced users in a variety of environments on an easy-to-use flow cytometry system, such as the Cytek® Muse® Micro cell analyzer.

The Cytek Muse Micro system utilizes microcapillary flow cytometry to enable users to obtain cell measurements quickly and reliably while maintaining a small footprint. The system provides absolute counts, percentages, and fluorescence intensity of populations while generating minimal biohazardous waste of less than 50 mL per eight-hour workday. This system only requires small volumes of whole blood or PBMC samples with no-wash assays, and provides flexibility for using pre-optimized Muse® reagent kits or user-defined assays. With Muse reagent kits, such as the Muse® Count & Viability kit or the Muse® Annexin V & Dead Cell kit, users can generate valuable data on cell health, concentration, and viability of immune cell samples. Furthermore, when employing Muse two-color reagent kits, phenotyping of T cell and B cell populations can be reliably performed. User-defined assays combining Cytek cFluor® and Tonbo® reagents with Muse® Micro InCyte™ software enable the system to support advanced and flexible immunophenotyping using fluorescently labeled CD markers, making it a versatile solution for routine and specialized immune analysis.

Cytek has a wide variety of commonly used clones for CD antigens with multiple fluorophore options, providing flexibility in assay design for the Cytek Muse Micro system. Available fluorophores include commonly used conjugates such as fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-Cyanine5 (PE-Cy5), and the cFluor equivalents cFluor® B520, cFluor® BYG575, and cFluor® BYG667, respectively. In addition, lysing solutions and compatible staining buffers are also available to support streamlined workflows.

Herein, we present specific protocols and example data generated to identify various immune cell populations using common phenotyping markers to analyze whole blood or PBMC samples on the Cytek Muse Micro system. Results generated with external blood controls demonstrate the accuracy of the system to provide absolute counts and population percentages.

Figure 1

Figure 1: Immunophenotyping analysis workflow on the Cytek Muse Micro system depicting simple sample preparation and examples of outputs for cell-based assays. **1A)** Simple sample staining protocol for assays performed on the Muse Micro system. **1B)** Example analysis and plot layout from the Muse Human CD4 T cell assay kit module. **1C)** A three-color assay acquired with open Muse Micro InCyte software.

Materials And Methods

Materials

All antibody conjugates, the staining buffer, and lysing solution were supplied by Cytek Biosciences. Antibodies conjugated with FITC, PE, PE-Cy5, cFluor B515, cFluor BYG575, or cFluor BYG667 fluorochromes were used in the studies described here. Part numbers of the reagents are provided in the table at the end of this document, and details for staining protocols are described below. CD-Chex Plus® and CD-Chex Plus CD4 Low immunophenotyping controls were purchased from Streck Laboratories. PBMCs were purified in-house using standard Ficoll separation methods for this study. Whole blood samples were obtained from Stanford Blood Center (Palo Alto, California).

Antibody Cocktail

For each stained sample, a 10 µL antibody cocktail was prepared using the Flow Staining Buffer (1X). To prepare the antibody cocktails, 0.25 µL to 2 µL of Cytek antibodies were added per sample per antibody and diluted to a final volume of 10 µL per sample using the Flow Staining Buffer (1X). For most antibodies used, 1 µL of antibody per sample showed optimal performance, however, titrations of some antibodies were needed, with a range of 0.25 µL to 2 µL used. Details on each antibody concentration evaluated are provided below.

Preparation Of CD4 Single Color Antibody Cocktails For CD-Chex Plus Controls Or PMBC Analysis

For CD4 staining, an antibody cocktail (40 µL total for 4 tests) was prepared by combining 36 µL of the Flow Staining Buffer (1X) with 4 µL of either cFluor® B515 Anti-Human CD4, cFluor® B532 Anti-Human CD4, cFluor BYG575 Anti-Human CD4, or cFluor BYG667 Anti-Human CD4. From this cocktail, 10 µL was used for each staining reaction.

Preparation Of Multi-Color Antibody Cocktail For Whole Blood And PBMCs

Two multi-color antibody cocktails were used for these studies. They were prepared as follows:

- To prepare two-color antibody cocktails comprising of CD3 and CD4 antibodies (40 µL total for 4 tests), 1 µL of PE-Cy5 Anti-Human CD3 (0.25 µL per test), and 4 µL of cFluor BYG575 Anti-Human CD4 (1 µL per test) were added to 35 µL of the Flow Staining Buffer (1X).
- To prepare a three-color cocktail comprising of CD3, CD4, and CD8 antibodies (40 µL total for 4 tests), 1 µL of PE-Cy5 Anti-Human CD3 (0.25 µL per test), 4 µL of cFluor BYG575 Anti-Human CD4, and 4 µL of cFluor B515 Anti-Human CD8 antibodies (1 µL per test) were added to 31 µL of the Flow Staining Buffer (1X) to bring the total volume to 40 µL. 10 µL of the prepared cocktail was used per staining reaction.

Preparation Of Multi-Color Antibody Cocktail For CD-Chex Plus Controls

Two multi-color antibody cocktails were used for these studies. They were prepared as follows:

- To prepare a three-color antibody cocktail for staining of blood controls comprising of CD45, CD3, and CD4 antibodies (400 µL total for 40 tests), 10 µL of FITC Anti-Human CD45 (0.25 µL per test), 10 µL of PE-Cy5 Anti-Human CD3 (0.25 µL per test), and 5 µL of PE Anti-Human CD4 (0.125 µL per test) antibodies were added to 375 µL of the Flow Staining Buffer (1X) to bring the total volume to 400 µL. The mixture was vortexed gently. 10 µL of the prepared cocktail was used per staining reaction.
- To prepare a three-color antibody cocktail for staining of blood controls comprising of CD3, CD4, and CD8 antibodies (400 µL total for 40 tests), 10 µL of PE-Cy5 Anti-Human CD3 (0.25 µL per test), 5 µL of PE Anti-Human CD4 (0.125 µL per test), and 10 µL of FITC Anti-Human CD8 (0.25 µL per test) antibodies were added to 375 µL of the Flow Staining Buffer (1X) to bring the total volume to 400 µL. 10 µL of the prepared cocktail was used per staining reaction.

Preparation Of RBC Lyse/Fix Solution (1X)

One part of Cytek® RBC Lyse/Fix Solution (10X) was diluted with nine parts of room temperature deionized water to prepare 1X RBC Lyse/Fix Solution before use.

Whole Blood Or CD-Chex Plus Control Staining Procedure

10 µL of whole blood, CD-Chex Plus, or CD-Chex Plus CD4 Low control was added to the staining tube. 10 µL of thoroughly mixed antibody cocktail was added to the sample. Samples were vortexed and incubated for 20 minutes at room temperature in the dark. Next, 380 µL of Cytek RBC Lyse/Fix Solution (1X) was added to the samples. Samples were vortexed and incubated for 15 minutes in the dark. Samples were then acquired on a Cytek Muse Micro system using Cytek Muse Micro InCyte software.

PBMC Staining Procedure

10 µL of PBMCs (in PBMC medium, at a concentration of 1.5 x 10^7 million cells/mL) was added to a sample tube. 10 µL of thoroughly mixed antibody cocktail was added next. Samples were vortexed and incubated for 20 minutes at room temperature in the dark. 380 µL of the Flow Staining Buffer (1X) was added to the tube and vortexed. Samples were then acquired on a Cytek Muse Micro system using Cytek Muse Micro InCyte software.

Sample Acquisition

All samples were acquired with Cytek Muse Micro InCyte software. Plots and counting gates were set up for optimal acquisition. In the examples shown, 3,000 CD45+ events, or 3,000 lymphocytes defined using FSC vs. SSC plots were collected. Gated event count may change depending on the panel being evaluated.

Results

Data of stained whole blood, CD-Chex Plus controls, and PBMC samples are shown in the figures below. These examples demonstrate the capability of the Cytek Muse Micro system to obtain useful immune cell sub-population data combined with one- to three-color assays and Muse Micro InCyte software.

CD4 Staining Of CD-Chex Plus Controls

Figure 2

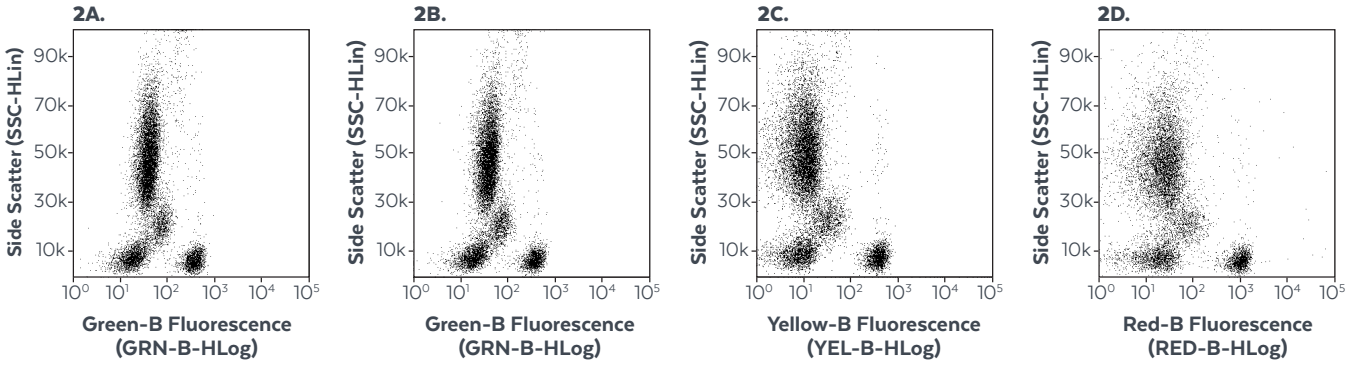

Figure 2: Staining profiles of CD-Chex Plus control labeled with Anti-Human CD4 antibodies conjugated to different cFluor reagents in a lyse no-wash assay and analyzed on a Cytek Muse Micro system. Fluorophore vs. SSC plots are shown above. Representative plots from samples **2A)** cFluor B515 Anti-Human CD4, **2B)** cFluor B532 Anti-Human CD4, **2C)** cFluor BYG575 Anti-Human CD4, and **2D)** cFluor BYG667 Anti-Human CD4.

CD4 Staining Of PBMC Samples

Figure 3

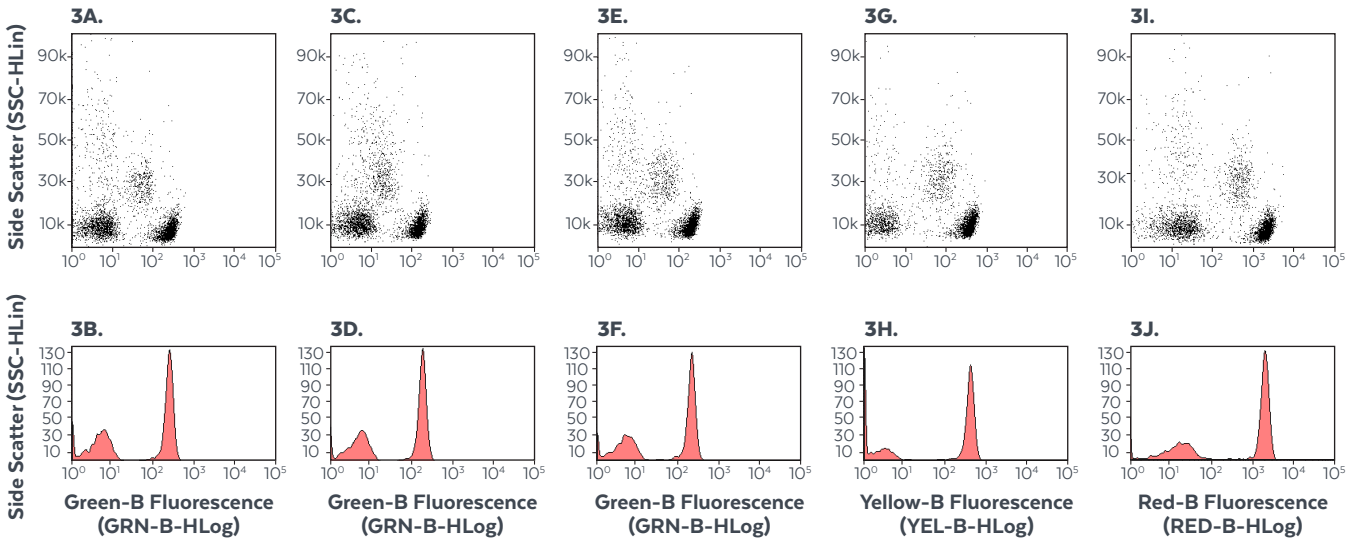

Figure 3: Staining profiles of PBMCs labeled with Anti-Human CD4 antibody conjugated to different cFluor reagents and analyzed on a Cytek Muse Micro system. Plots **3A, 3C, 3E, 3G, and 3I** represent fluorophore vs. SSC. Plots **3B, 3D, 3F, 3H, and 3J** compare samples gated on the lymphocyte population from FSC vs. SSC plots (data not shown). Plots for each fluorochrome are as follows: **3A)** and **3B)** cFluor B515 Anti-Human CD4; **3C)** and **3D)** cFluor B520 Anti-Human CD4; **3E)** and **3F)** cFluor B532 Anti-Human CD4; **3G)** and **3H)** cFluor BYG575 Anti-Human CD4; **3I)** and **3J)** cFluor B667 Anti-Human CD4.

Two-Color Staining Of CD3 And CD4 With Whole Blood

Figure 4

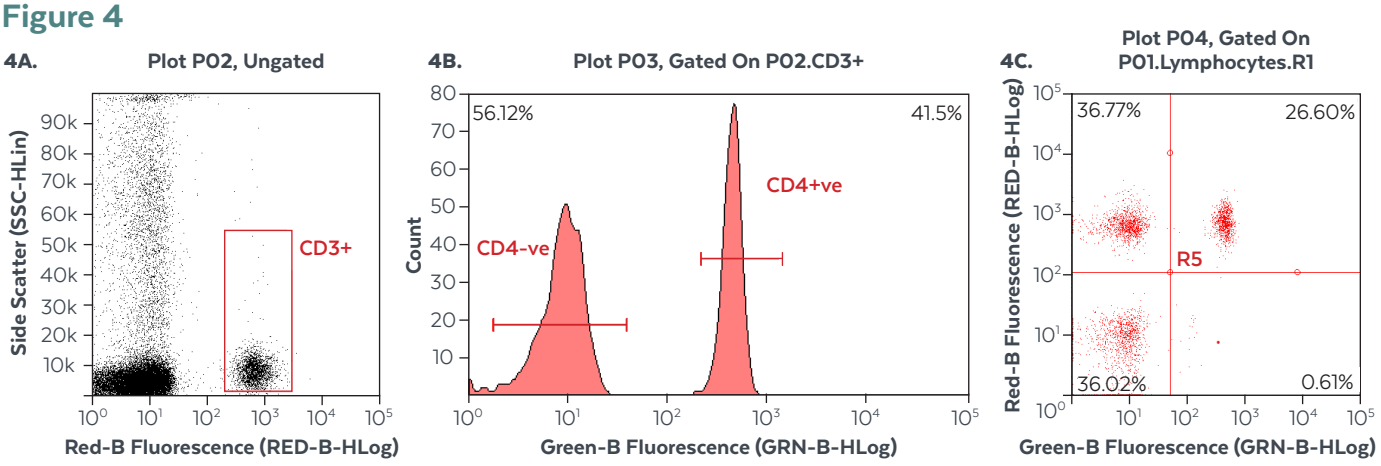

Figure 4: Representative data of whole blood stained with PE-Cy5 Anti-Human CD3 and cFluor B515 Anti-Human CD4 antibody cocktail in a lyse no-wash assay and acquired on a Cytek Muse Micro system. **4A)** SSC vs. PE-Cy5 Anti-Human CD3 (Red-B) was plotted and CD3+ events were gated followed by **4B)** CD3+ cells displayed on a histogram displaying cFluor B515 Anti-Human CD4 fluorescence gated on positive and negative CD4 cells. **4C)** A dot plot of CD3 vs. CD4 gated on the lymphocyte population from FSC vs. SSC (plot not shown).

3-Color Staining Of PMBC Samples

Figure 5

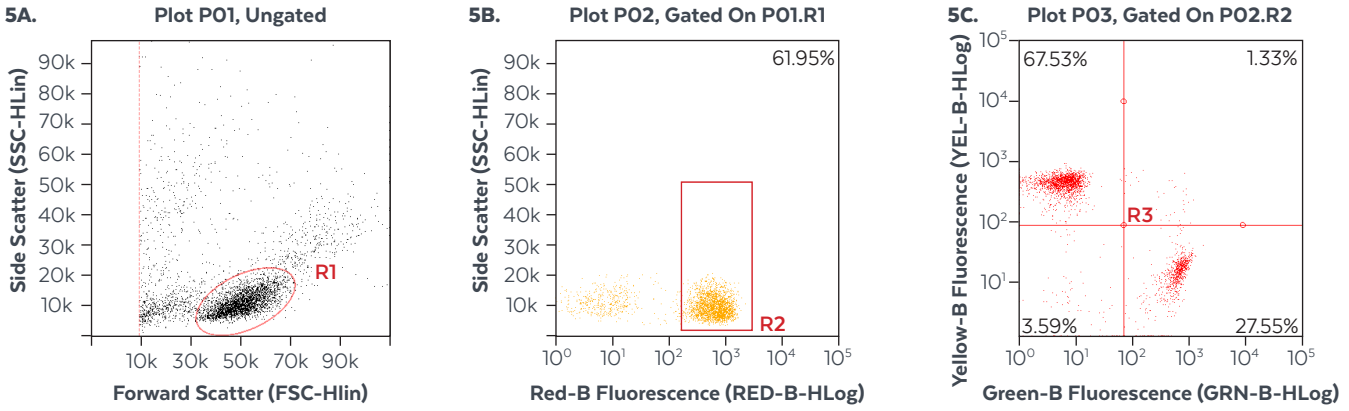

Figure 5: Expression profiles of PBMCs stained with PE-Cy5 Anti-Human CD3, cFluor BYG575 Anti-Human CD4, and cFluor B515 Anti-Human CD8 antibody cocktail in a no-wash staining procedure and analyzed on the Cytek Muse Micro system. **5A)** Lymphocytes were gated on the FSC vs. SSC plot followed by a plot displaying **5B)** CD3 (RED-B) vs. SSC to identify CD3+ events. **5C)** CD3+ cells were gated and subsets determined with a CD8 (GRN-B) vs. CD4 (YEL-B) plot.

Quantification Of CD3, CD4, And CD8 T Cells With CD-Chex Plus Controls

Figure 6

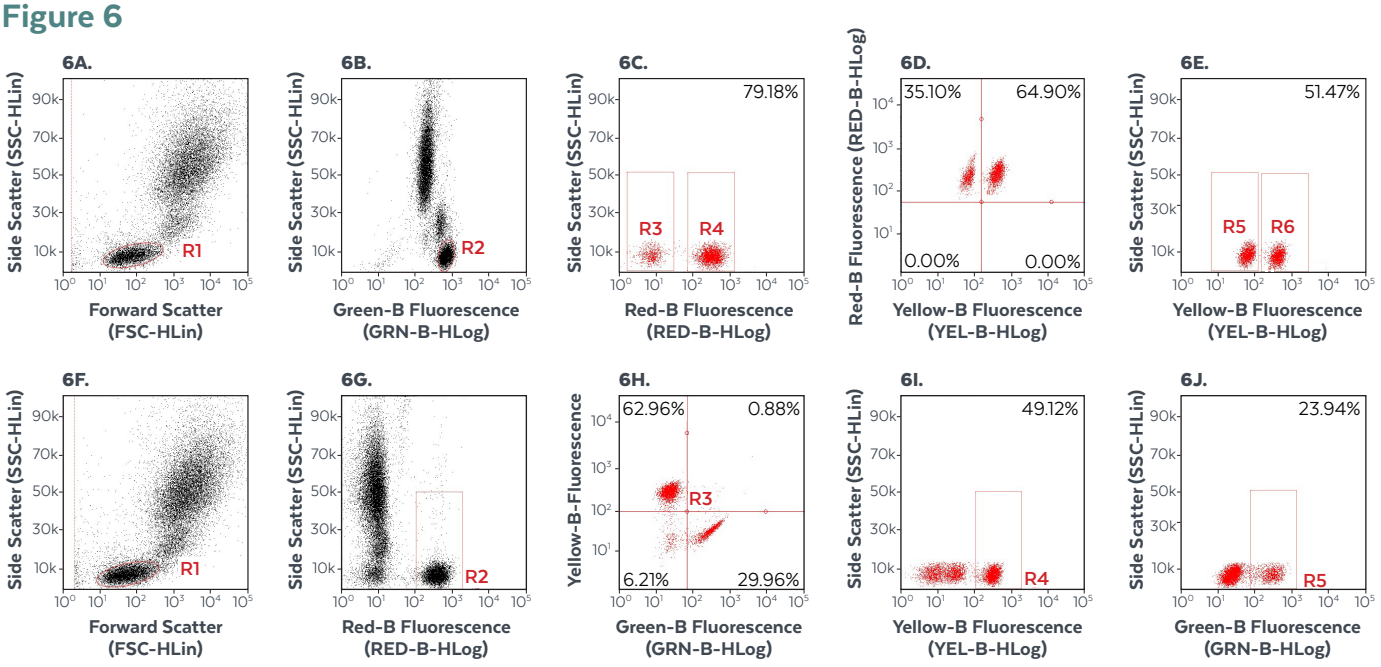

Figure 6: Example data of T cell subset analysis from the Cytek Muse Micro system using CD-Chex Plus controls stained with either a FITC Anti-Human CD45, PE-Cy5 Anti-Human CD3, and PE Anti-Human CD4 antibody cocktail **6A-6E)** or with a PE-Cy5 Anti-Human CD3, PE Anti-Human CD4, and FITC Anti-Human CD8 antibody cocktail **6F-6J)**. For samples stained with CD45, CD3, and CD4; lymphocyte populations were identified from the **6A)** FSC vs. SSC plot and displayed on a **6B)** SSC vs. CD45 plot. CD45+ cells were then gated into a **6C)** SSC vs. CD3 plot and gated on the CD3+ cells. Evaluation of CD4 cells was performed using a **6D)** CD3 vs. CD4 plot and **6E)** SSC vs. CD4 to identify CD4+ cells. For samples stained with CD3, CD4, and CD8; lymphocytes were identified using a **6F)** FSC vs. SSC plot and displayed on a **6G)** SSC vs. CD3 plot. Positive CD3 cells were gated into a **6H)** CD4 vs. CD8 plot to determine CD4 and CD8 positive cells. Additionally, **6I)** SSC vs. CD4 and **6J)** SSC vs. CD8 plots were evaluated.

Figure 7

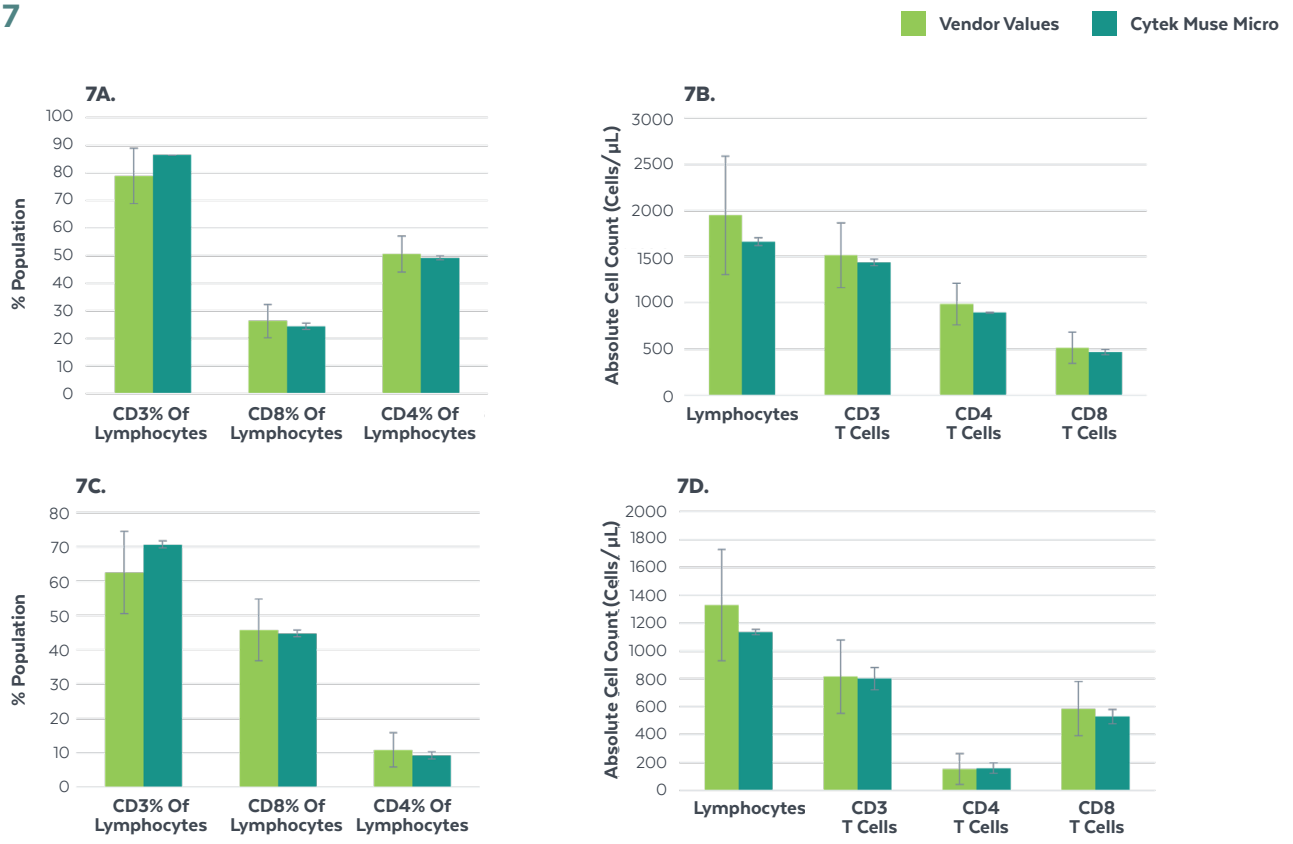

Figure 7: Quantitative analysis of T cell percentages and absolute cell counts on the Cytek Muse Micro system. CD-Chex Plus and CD-Chex Plus CD4 Low controls were stained with a PE-Cy5 Anti-Human CD3, PE Anti-Human CD4, and FITC Anti-Human CD8 antibody cocktail, in a lyse no-wash assay and analyzed on the Cytek Muse Micro system. Results of population percentages and absolute counts from the system were compared against vendor (Streck) provided values in the bar graphs above for CD-Chex Plus control **7A)** and **7B)** and CD-Chex Plus CD4 Low control **7C)** and **7D)**. The Cytek Muse Micro system results display the average concentration for triplicate samplings. The error bars represent standard deviations. The vendor labeled results represent the vendor target values, while the bars show the upper and lower limits. These results demonstrate that the Cytek Muse Micro system values closely match the vendor target values and fall within accepted ranges.

Conclusion

One- to three-color immunophenotyping can be easily performed on the Cytek Muse Micro system using Cytek reagents, simple no-wash protocols, and low volumes of blood or PBMC samples. The simplicity of data collection for both absolute cell counts and percentages of immune cell sub-populations from the Cytek Muse Micro system provides utility for a wide range of studies. Additionally, the availability of reagents from Cytek including antibody conjugates for commonly used clones, as well as lysing solutions and staining buffers, further streamline analyses to support diverse research applications.

The table on the following page lists the CD markers used in these studies on the Cytek Muse Micro system. Additional clones, antigens, and fluorochromes are available on the Cytek website.

Description	Part Number
FITC Anti-Human CD45 (HI30)	35-0459
PE-Cyanine5 Anti-Human CD3 (UCHT1)	55-0038
PE Anti-Human CD4 (SK3)	50-0047
FITC Anti-Human CD8 (SK1)	35-0087
cFluor® BYG575 Anti-Human CD4 (SK3)	R7-20156
cFluor® B515 Anti-Human CD4 (SK3)	R7-20028
cFluor® B520 Anti-Human CD4 (SK3)	R7-20150
cFluor® B532 Anti-Human CD4 (SK3)	R7-20038
cFluor® BYG575 Anti-Human CD4 (SK3)	R7-20156
cFluor® BYG667 Anti-Human CD4 (SK3)	R7-20158
Cytek® RBC Lyse/Fix Solution 10X	R7-60010
Flow Staining Buffer (1X)	TNB-4222-L500

For Research Use Only. Not for use in diagnostic procedures.

cFluor® B515 and cFluor® B532 are equivalent to CF®488A and CF®503R, respectively, manufactured and provided by Biotium, Inc. under an Agreement between Biotium and Cytek (LICENSEE). The manufacture, use, sale, offer for sale, or import of the product is covered by one or more of the patents or pending applications owned or licensed by Biotium. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel.

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