NovoCyte™ Flow Cytometer

Application Note No. 4

July 2014

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10-Color Analysis of T Cell Subpopulations

Introduction

Flow cytometry is an essential tool for analysis of multiple qualitative and quantitative characteristics of a single cell in a mixed cell population. It is widely used in life science research, drug discovery and plant and agricultural science research to assess cell cycle, cell proliferation, and DNA content. It is also increasingly applied in routine clinical laboratories for the diagnosis, prognosis and monitoring of diseases. The NovoCyte flow cytometer offers a combination of features, including powerful signal detection, intuitive user friendly software, direct volumetric cell count, and flexible configurations for many application including analysis of the human immune system.

Understanding the function and interaction of the molecular and cellular components of the immune system demands multiparameter analysis. The NovoCyte flow cytometer, with its innovative optical detection design, can simultaneously detect up to 15 parameters in a single experiment, enabling identification and detailed study of subpopulations of cells in blood samples. Blood is comprised of complex populations of lymphocyte, monocyte, granulocyte, platelet and red blood cells. Flow cytometry represents the best method for studying functional and phenotypic properties of these sub-populations based on biological function and cell-surface antigen expression.

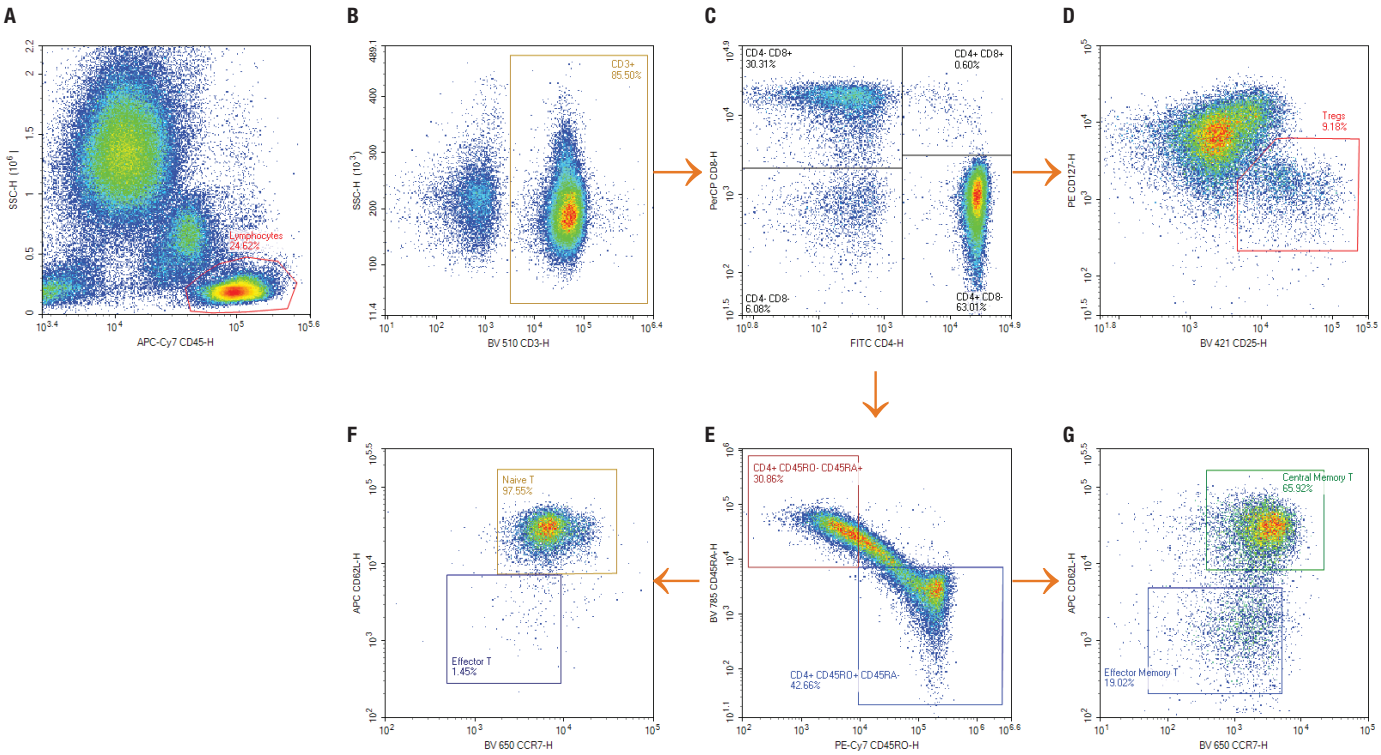

Figure 1. CD4⁺ cell subpopulations analysis: Normal human whole blood was stained with a cocktail of 10 mAbs (see Table 1 on next page). Cells were analyzed on ACEA NovoCyteTM 3000 (Cat#2010011). In this figure, $CD4^+$ T lymphocytes (lower right quadrant of Plot C) were further analyzed. $CD4^+$ T_{reg} cells express high levels of CD25 and low levels of CD127 (Plot D). The $CD45RO^-RA^+$ (upper left quadrant of Plot E) population generally represents naïve/effector $CD4^+$ T cells, which can be further divided into naïve T and effector T cells based upon different levels of CD62L and CCR7 expression (Plot F). $CD45RO^+RA^-$ cells are mainly activated T cells and memory T cells (lower right quadrant of Plot E). The memory T cells can be further divided into central memory T cells and effector memory T cells by analyzing expression levels of CD62L and CCR7 (Plot G).

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Antibody Specificity	Format	Laser (Excitation)	FL Channel on NovoCyte	Filter (Emission)
CD25	BV421	405nm	VL1	445/45
CD3	BV510	405nm	VL2	530/30
CCR7	BV650	405nm	VL5	675/30
CD45RA	BV785	405nm	VL6	780/60
CD4	FITC	488nm	BL1	530/30
CD127	PE	488nm	BL2	585/40
CD8	PerCP	488nm	BL4	675/30
CD45RO	PE-Cy7	488nm	BL5	780/60
CD62L	APC	640nm	RL1	675/30
CD45	APC-Cy7	640nm	RL2	780/60

Table 1. Expanded Detection Channel Capabilities with 3-Laser System: This table summarizes the 10-color experiment antibody combination using the NovoCyte three laser system which includes 4 colors of the 405nm laser, 4 colors of the 488nm laser and 2 colors of the 640nm laser.

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For more information, visit www.aceabio.com or email info@aceabio.com

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