

"Go with the Flow..."

Cytometry Research, LLC

"...Flow Cytometry, that is!"

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FACS Quantification of Lymphocyte Subsets

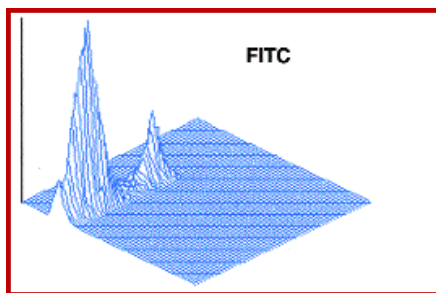
Immunophenotyping by flow cytometry in both mouse and human research applications has shown a sharp increase in recent years. The ability to assess the immune response within the body as it exists in normal individuals and also in individuals subjected to some treatment or other physical challenge has given researchers a vast amount of knowledge.

This unique application utilizes monoclonal antibodies labeled with fluorescent dyes. The specific antigens defined by these antibodies make up the lymphocyte cell surface phenotypes. They facilitate the quantitation of T cells, B cells and natural killer cells, as well as subsets of these populations.

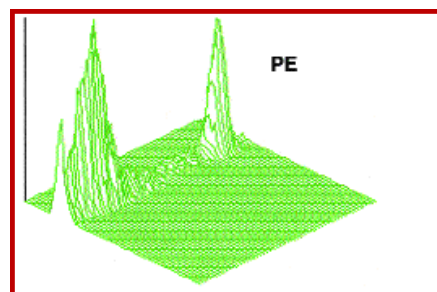
In the interest of standardization, the Center for Disease Control and Prevention has approved a set of eight antibodies with specific cluster designations (CD) for the identification of subsets of lymphocytes. The two

markers used to identify leukocytes are CD45 and the monocyte marker CD14. Subsets of T cells can be identified using CD4 and CD8. B lymphocytes are identified using CD19. Natural killer (NK) lymphocytes can be identified using a combination of CD3 and CD16 or CD3 and CD56.

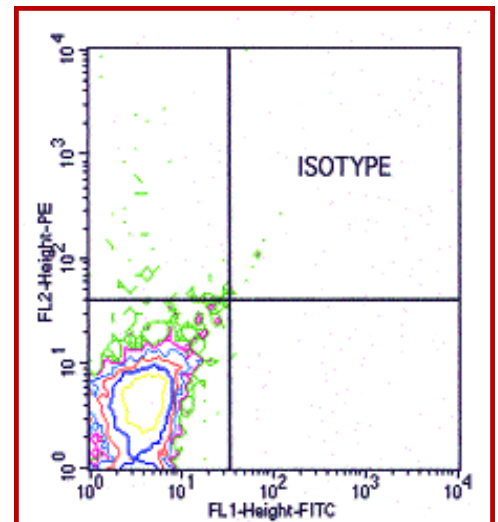
The ability to identify and quantitate specific lymphocyte subsets has enabled scientists to locate deficiencies in the immune system response and to identify lymphocyte subset differentiation and abnormalities based on their comparison with normal individuals. Flow cytometry has played a key role in helping to standardize and to expand the scope of this important research and diagnostic tool. FACS (fluorescence-activated cell sorting) has the added capability of separating these subsets based on their fluorescent cell surface receptors. Purities of greater than 95% can be easily achieved using cell sorting.



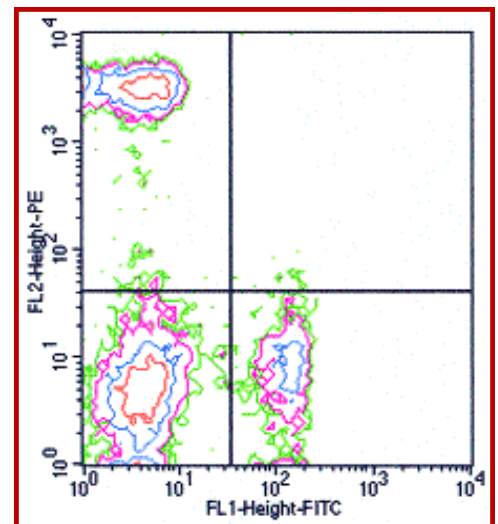
A two-parameter frequency histogram demonstrating the staining profile of CD4 FITC-positive T-cells. The taller peak represents the negative population while the smaller peak represents the positive cells.



A two-parameter frequency histogram of CD8 PE-positive lymphocytes. Each peak height corresponds to the relative cell numbers within that subpopulation.



A bivariate contour graph showing an isotype control with no staining evident in any other quadrant.



A bivariate contour graph showing T-cell lymphocytes stained with PE and FITC bound to monoclonal antibodies against CD4 and CD8 antigens, respectively. Quadrant markers aid in the quantitation of CD4- and CD8-positive events. Log fluorescence is plotted on both axes.

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Denise Higgins, Editor

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