

"Go with the Flow..."

Cytometry Research, LLC

"...Flow Cytometry, that is!"

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Cell Characterization by Flow Cytometry

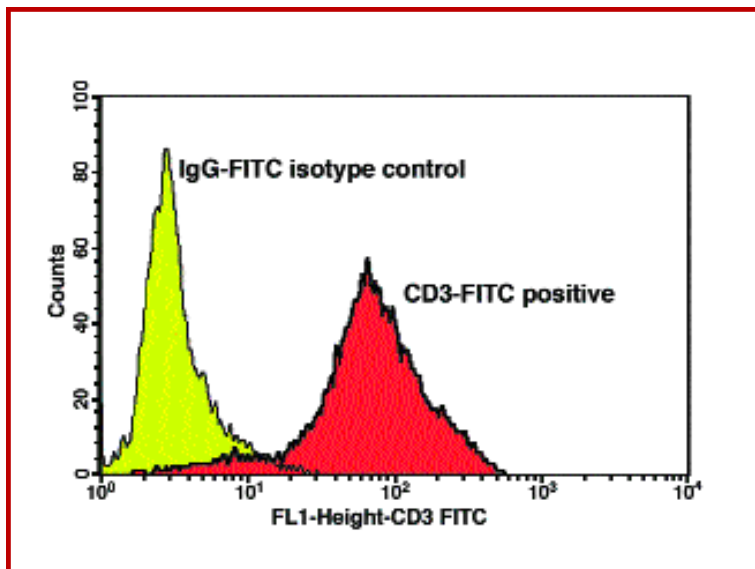
Flow cytometry employs instrumentation that scans single cells flowing past excitation sources in a liquid medium. The technology can provide rapid, quantitative, multiparameter analyses on single living (or dead) cells based on the measurement of visible and fluorescent light emission. Flow cytometry is a widely used method for characterizing and separating individual cells.

The most common application in flow cytometry is direct staining with fluorochrome-conjugated antibodies against specific cell markers. So, if you want to find out if your cells express CD3, you can use a CD3-FITC antibody conjugate. When your samples are analyzed using flow cytometry, the results will show how specific and active the CD3 antibody is to your cells. In flow cytometry, fluorescence is relative. You need a negative control to determine where the positive results begin. In this case, the cells that you believe to be expressing CD3 are your positive control.

You can provide a negative control in a couple of different ways. You can use a fluorochrome-conjugated antibody of the same immunoglobulin isotype but to an irrelevant antigen. Or, you can use the same fluorochrome-conjugated antibody (CD3-FITC in our example) on cells that are known not to express the antigen.

On page 2 of this issue is a basic protocol for use with direct-conjugated antibodies. This is the protocol most often used by Cytometry Research customers and a good way for anyone to get started in flow cytometry. You can visit our website for more protocols and links to other helpful sites.

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This histogram demonstrates the characterization of mature T-lymphocytes flow cytometry. CD3-FITC was used to label the T-cells that positively expressed the CD3 cell surface marker. An IgG-FITC isotype control was used to measure background fluorescence as a negative control for the CD3 antibody. The isotype control binds non-specifically to IgG and provides a basis for comparison to those cells that were labeled with CD3-FITC.

The shift to the right of the CD3-FITC positive peak indicates that specific binding has occurred between those cells expressing the CD3 marker and the CD3 antibody conjugated to the fluorescent dye. The fact that the entire peak has shifted dramatically to the right indicates that almost all the cells express at least some CD3 marker. The right edge of the positive peak represents those cells that possess the highest number of the CD3 marker, while those at the left edge of the peak possess fewer. This type of shift is proof of a very ubiquitous and high level of CD3 expression in the tested cell line.

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