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Jackson ImmunoResearch LABORATORIES INC.

SECONDARY ANTIBODIES FOR

Flow cytometry

Flow Cytometry

with Jackson ImmunoResearch

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Flow cytometry is a powerful technique for measuring and analyzing the physical characteristics of single particles in solution as they travel past a beam of light. Properties such as relative size and fluorescence can be measured, and fluorescently tagged antibodies enable cells to be interrogated for multiple proteins and molecular dynamics.

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INDIRECT

Jackson ImmunoResearch LABORATORIES INC.

Flow Cytometry

Indirect for signal amplification Flow cytometry can be performed directly, using conjugated primary antibodies, or indirectly, using a conjugated secondary antibody to bind an unconjugated primary. Indirect flow cytometry allows the choice of a wide range of probe molecules, enabling the user to match the desired probe with any primary antibody. Secondary antibody conjugates can improve a flow cytometry experiment by preserving the active site of the primary antibody, and by signal amplification.

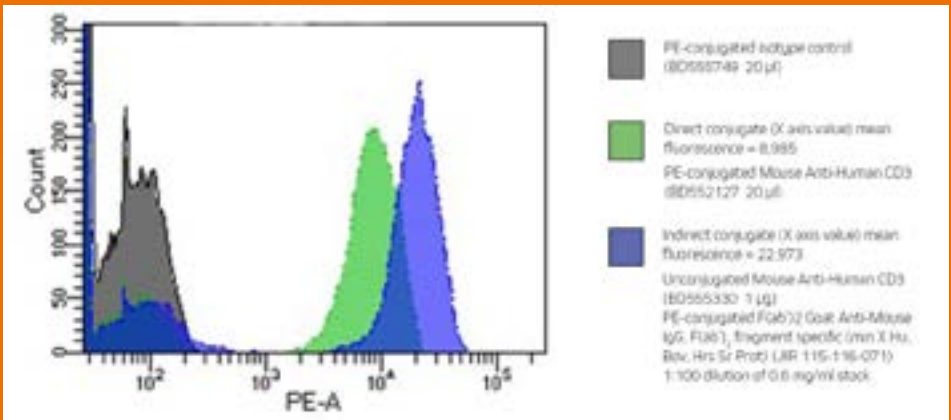

Figure 1. Comparison of direct and indirect flow cytometry methods. Human peripheral blood gated lymphocytes after ammonium chloride lysis of erythrocytes were analyzed for CD3 expression using direct or indirect flow cytometry. Comparison of mean fluorescence showed that the indirect method produced a brighter signal (22,973) compared to the direct method (8,985). (Experiment performed on BD FACSCelesta).

Secondary Antibody Format for Flow Cytometry

Alongside whole molecule IgG antibodies, F(ab')$_2$ and fab fragment antibodies are available. Choosing the correct format can impact the success of an experiment, F(ab')$_2$ fragments are divalent fragments comprised of two Fab arms and no Fc domain. When used to stain tissue or cells, the absence of Fc can help to avoid background caused Fc receptors expressed on cell surfaces which lead to off-target binding. FabuLight™ secondary antibodies are monovalent Fab fragments specific for the Fc region of primary antibodies, preventing interaction with the primary antibody's antigen-binding region. Conjugated FabuLights are convenient for labeling primary antibodies prior to incubation with an experimental sample, saving incubation and wash steps. Like F(ab')$_2$ fragments FabuLights can minimize background staining by preventing Fc receptor binding.

Fluorescent conjugates for flow cytometry

Fluorescent dyes from across the spectrum can be used for flow cytometry, depending on instrument capabilities. JIR conjugates a wide range of fluorescent dyes, including Alexa Fluor, Brilliant Violet, Cyanine, and three large, bright fluorescent proteins (R-PE, APC, and PerCP), making it possible to design a flow cytometry dye panel that accommodates instrument capabilities and experimental needs.

Controls for Flow Cytometry

Controls are essential to validate an experiment and interpret results. ChromPure™ proteins are purified from the serum of non-immunized animals and are appropriate experimental controls. An isotype control is a negative control that can be used to estimate the non-specific binding of an antibody. Isotype controls are antibodies that match the host species and class of antibodies used in the experiment but are not directed against the antigen of interest. An isotype control should be conjugated with the same reporter molecule as the specific antibody.

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