R&D SYSTEMS®

Intracellular Staining Reagents

Anti-human IL-5-Phycoerythrin Monoclonal Antibody

Catalog Number: IC605P
Lot Number: AAHZ01
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated anti-human IL-5: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone: 9906

Ig class: mouse IgG_1

Storage: Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Additional Reagents Required

Paraformaldehyde Fixative - Dissolve 4.0 g of paraformaldehyde in 100 mL of sterile PBS (10 mM phosphate buffered saline, pH 7.4) by heating the solution at 56° C for about 1 hour. All solids must be fully dissolved prior to use. Store buffer at 2° - 8° C, protected from light, for no longer than 2 weeks.

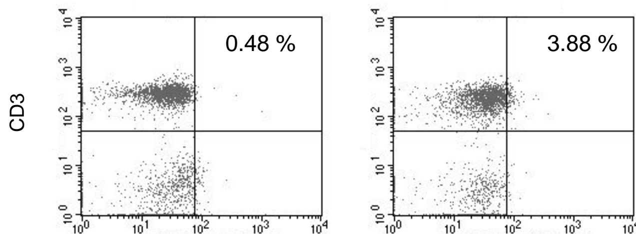
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN_3 in Hanks' Balanced Salt Solution (HBSS). Store at 18° - 24° C for no longer than 1 month.

Intended Use

The reagent is designed for flow cytometric applications intended to identify and quantitate cells possessing cytoplasmic forms of the protein recognized by the monoclonal.

Principle of the Test

Fixed cells are permeabilized, allowing conjugated antibodies access to proteins within the cell. Cells are initially fixed in order to minimize leakage of proteins out of the cell. The conjugated antibody is allowed to penetrate and bind to its target within the cell. Following a final wash, the cells are analyzed on a flow cytometer. Flow cytometric analysis of PE conjugates will generate a signal, which can be detected using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector, optimized to collect peak emissions at 565 - 605 nm.



Intracellular staining of resting (left) and PMA/ionomycin-activated (right) human PBMCs with PE-conjugated anti-human IL-5 (Catalog # IC605P) and APC-conjugated anti-human CD3 (Catalog# FAB100A). Quadrant markers are set based on isotype control staining (Catalog # IC002P).

Sample Preparation

Intracellular staining antibodies are designed for multiparameter flow cytometric analysis of cells. If it is desirable to stain for surface proteins, such as CD3, CD4, CD8, etc., in addition to the intracellular staining properties of the cells, we recommend that the investigator determine whether the fixation and permeabilization steps adversely affect the surface protein. Should this be the case, surface staining of cells prior to fixation and permeabilization is recommended.

For intracellular staining, the cells must first be fixed and permeabilized. The use of 4% paraformaldehyde in PBS as a fixative is recommended. Other formulations or tissue fixatives may affect the staining properties of the monoclonal antibody. For permeabilization, 0.1% saponin in a balanced salt solution is effective in facilitating antibody entry into the cells. Due to the reversible nature of cell membrane permeabilization, saponin **must** be included in all buffers used in both the staining as well as washing steps.

Sample Staining

1. Harvest the cells and wash twice in cold HBSS or PBS by spinning at 200 x g for 7 minutes.
2. If cells are to be surface stained, follow the staining procedure indicated by the manufacturer.
3. Resuspend a maximum of 5 x 10^5 washed (or surface stained) cells in 0.5 mL of cold 4% paraformaldehyde fixative and incubate at 18° - 24° C for 10 minutes.
4. Vortex the cells should be intermittently in order to maintain a single cell suspension. Following fixation, wash the cells twice in HBSS or PBS by centrifuging at 200 x g for 7 minutes.
5. Harvest the cell pellet from each tube and resuspended in 2 mL of SAP buffer.
6. Centrifuge the cells at 200 x g for 7 minutes.
7. Decant the supernate, ensuring that approximately 200 µL of SAP buffer remains in the tube.
8. Gently resuspend the cells in the remaining SAP buffer and add 10 µL (or a previously titrated amount) of antibody conjugate.
9. Briefly vortex the tube and incubated for 30 - 45 minutes at 18° - 24° C **in the dark**.
10. Wash the cells twice using 2 mL of SAP buffer each time. Centrifuge as in step 6.
11. Resuspend the cells in each tube with 200 - 400 µL of PBS for final flow cytometric analysis.

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Background Information on Intracellular Staining

The presence or absence of distinct cytokines determines the quality and quantity of the immune response following antigenic challenge (1, 2). Immune responses range from a cellular (macrophage/cytotoxic T cell) response (3 - 5), to a predominantly humoral or antibody-mediated immunity (6). IL-2, IL-12, IFN-γ and TNF-β are associated with cellular immunity (7 - 9), while IL-4, -5, -6, and -10 tend to accompany antibody responses (10 - 12). IL-2, IL-12, IFN-γ and TNF-β are known as type 1 cytokines. IL-4, -5, -6, -10 and -13 are referred to as type 2 cytokines (13).

T helper cells (Th1 and Th2) are considered end products of $CD4^+$ T cell differentiation. Th1 cells secrete IL-2, TNF-β and IFN-γ. Th2 cells generate IL-4, -5, -6, and -10. $CD4^+$ precursors are referred to as Thp or Th0 cells, since they secrete a combination of both type 1 and type 2 cytokines (14). Although this original Th1/Th2 model has been a tool for defining complex T cell interactions, it is now apparent that some modifications may be required. For example, Th1 and Th2 cytokines are not mutually exclusive, they can occur in many combinations (IL-5 with IFN-γ being a notable exception) (14 - 16). In addition, $CD4^+$ T cells are not the exclusive source of cytokine(s) for various effector cells, $CD8^+$ (or cytotoxic) T cells can also perform similar functions (17, 18). Type 1 cytokines can be generated from either Th1/$CD4^+$ or Tc1/$CD8^+$ T cells. Type 2 cytokine production is associated with either Th2/$CD4^+$ or Tc2/$CD8^+$ T cells (18). The ability to investigate cytokine expression at the single cell level, by combining the power of flow cytometry and intracellular cytokine staining reagents, may offer new insights into immunity (14, 15).

References

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Technical Notes

Appropriate negative controls should be used to verify specificity and rule out background staining. An irrelevant antibody of the same isotype and concentration (that does not stain mouse cells) should be tested to set quadrant statistics. Antibody binding can also be blocked with a pre-incubation of excess cytokine (10X molar excess should give > 90% inhibition of staining). In some cases, however, enhanced staining has been observed. This may be due to detection of cytokine binding cell surface receptors. Unlabeled antibody may also be used as a control. An excess of unlabeled antibody in the presence of labeled antibody should completely inhibit staining.

Cytokine production is usually the end result of cellular activation. The investigator is advised to determine which activation strategies and cytokine synthesis kinetics may yield optimal results. For example, IL-4 production may be elicited from T cells with the following stimulation protocol: ficolled human peripheral blood mononuclear cells or $CD4^+$ enriched T cells should be first cultured on anti-CD3 coated plates (5 - 10 μg/mL of anti-CD3 monoclonal, Catalog # MAB100, on tissue culture plates or flasks). After a 2 - 4 hour absorption step, excess anti-CD3 is washed from the plates with sterile PBS and the cells may be plated at a concentration of 1 - 2 million/mL. The cells are cultured for 2 days in the presence of 1 μg/mL of anti-human CD28 (Catalog # MAB342), 10 ng/mL of rhIL-2 (Catalog # 202-IL) and rhIL-4 (Catalog # 204-IL), and 2 μg/mL of anti-human IL-12 (Catalog # MAB219). On day 3, the cells are collected and fresh media is replaced along with fresh IL-2, IL-4 and anti-human IL-12. The cells are cultured for an additional 3 days. At the end of the culture period, the cells are exposed to a brief stimulation with PMA (50 ng/mL), calcium ionomycin (500 ng/mL) and monensin (3 μM) for 6 hours. The cells are harvested and processed as indicated above for intracellular cytokine staining.

Production of cytokines varies between different populations of cells as well as among cells within the same population. Some cells may produce a cytokine at concentrations below the detection limits of the monoclonal antibody. The investigator is encouraged to determine optimal titers for each antibody conjugate. The use of either monensin or brefeldin A, which act to block intracellular protein transport and result in an accumulation of protein in the Golgi, will enhance detection of cytokines in low level secreting cells.

Some antibodies are sensitive to the conformational structure of their target epitope. Fixation of cells using extremely harsh conditions or for a prolonged period of time may alter the target epitope and render it unrecognizable to the antibody. Strict attention to the recommended fixation conditions must be followed in order to generate consistent results.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.