Intracellular Staining Reagents

IL-1α-Fluorescein Monoclonal Antibody

Catalog Number: IC200F

Lot Number: LHT02

100 Tests

Reagent Information

Carboxyfluorescein (CFS)-conjugated anti-IL-1α: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone: 4414

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

Specificity: rhIL-1α

Ig class: mouse IgG_{2A}

Intended Use

The reagent is designed for flow cytometric applications intended to identify and quantitate cells expressing cytoplasmic forms of human IL-1α.

Principle of the Test

Fixed cells are permeabilized, allowing conjugated antibodies access to proteins within the cell. Cells are initially subjected to a fixation step in order to minimize leakage of proteins out of the cell. The conjugated antibody is allowed to penetrate and bind to its target cytokine within the cell. Following a final wash, the cells are analyzed on a flow cytometer. Flow cytometric analysis of carboxyfluorescein conjugates will generate a signal that can be detected in the FITC signal detector (usually FL1).

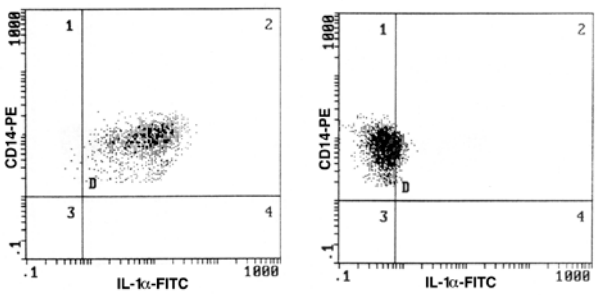
Additional Reagents Required

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 4° 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 room temperature for no longer than 1 month.

Typical anti-IL-1α staining of LPS-stimulated monocytes.
A) CD14-gated monocytes stained with IC200F. B) Inhibition of IC200F staining by the addition of excess IL-1α.

A **B**



Sample Preparation

Intracellular staining antibodies are designed for multiparameter flow cytometric analysis of cells. To stain for surface proteins (*e.g.* CD3, CD4, CD8, CD14) in addition to the intracellular cytokine, we recommend that the investigator determines whether the surface protein is adversely affected by the fixation and permeabilization steps. If so, surface staining of cells prior to fixation and permeabilization is recommended.

Prior to intracellular cytokine staining, cells must be fixed and permeabilized. 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 cells. Due to the reversible nature of cell membrane permeabilization, saponin **must** be included in all buffers used (both the staining and washing steps).

Sample Staining

1. Harvest 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 antibody 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. Cells should be vortexed intermittently in order to maintain a single cell suspension. Following fixation, cells are washed twice in HBSS or PBS by spinning at 200 x g for 7 minutes.
5. The cell pellet from each tube is harvested and resuspended in 2 mL of SAP buffer.
6. Cells are centrifuged at 200 x g for 7 minutes.
7. The supernate is decanted ensuring that approximately 200 μL of SAP buffer is left in the tube.
8. Cells are gently resuspended in the remaining SAP buffer and 10 μL (or a previously titrated amount) of antibody conjugate is added to each tube.
9. The tube is vortexed briefly and then incubated for 30 - 45 minutes at room temperature **in the dark**.
10. Each tube of cells is washed twice using 2 mL of SAP buffer each time (use same spinning instructions as in step 6).
11. Wash the cells one time in PBS by centrifugation at 200 x g for 7 minutes.
12. Resuspend the cells in each tube with 200 - 400 μL of PBS for final flow cytometric analysis.

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1-800-343-7475

Background Information

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 run to verify specificity and rule out background staining. An irrelevant antibody of the same isotype and concentration (that does not stain human cells) should be run to set quadrant statistics. Antibody binding can also be blocked with a preincubation 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 the 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. Monocyte activation has been performed by culturing ficolled peripheral blood mononuclear cells (5×10^6 cells/mL) with 1 μg/mL of LPS for 24 hours in the presence of 2 μM monensin.

Production of cytokines varies between different populations of cells as well as among cells of 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, may enhance detection of cytokines in low level secreting cells. To distinguish between detection of cell surface-bound *vs*. intracellular cytokines, we recommend preincubating cells with the same monoclonal antibody in an unconjugated form prior to fixation, permeabilization and staining with the fluorochrome-conjugated anti-cytokine antibody.

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.