Intracellular Staining Reagents

IL-8-Fluorescein Monoclonal Antibody
Catalog Number: IC208F
Lot Number: LIE04
100 Tests

Reagents Information

Carboxyfluorescein (CFS)-conjugated anti-IL-8: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone: 6217

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

Specificity: rhIL-8

Ig class: mouse IgG_1

Intended Use

The reagent is designed for flow cytometric applications intended to identify and quantitate cells expressing cytoplasmic forms of human IL-8.

Principle of the Test

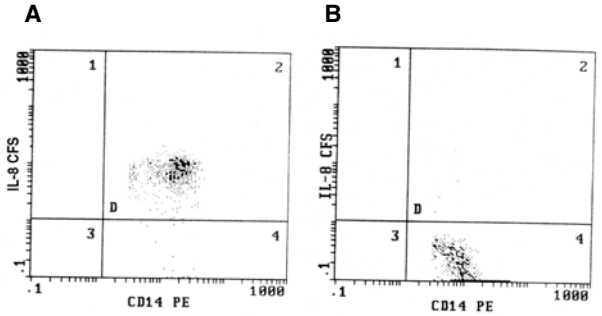
Fixed cells are permeabilized, allowing conjugated antibodies access to proteins within the cell. Cells are initially subjected to a fixation step in order to minimize leakage of proteins out of the cell. The conjugated antibody is allowed to penetrate and bind to its target cytokine within the cell. Following a final wash, 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 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 room temperature for no longer than 1 month.

Typical anti-IL-8 staining of LPS-stimulated monocytes. A) CD14-gated monocytes stained with IC208F. B) Inhibition of IC208F staining by the addition of excess IL-8.



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.

For intracellular cytokine staining, cells must first 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 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. A maximum of 5 x 10^5 washed (or surface stained) cells should be resuspended in 0.5 mL of cold 4% paraformaldehyde fixative and incubated at room temperature 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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

Chemokines constitute a superfamily of small, inducible, secreted cytokines that are involved in a variety of immune and inflammatory responses, acting primarily as chemoattractants and activators of specific types of leukocytes.[1, 2] Four classes of chemokines have been defined by the arrangement of the conserved cysteine (C) residues of the mature proteins: the C-X-C chemokines that have one amino acid residue separating the first two conserved cysteine residues; the C-C chemokines in which the first two conserved cysteine residues are adjacent; the C chemokines which lack two (the first and third) of the four conserved cysteine residues; and C-X_3-C chemokines which have three intervening amino acid residues between the first two conserved cysteine residues.[3]

C-X-C chemokines have been shown to be chemotactic for neutrophils and lymphocytes. By contrast, the C-C chemokines were found to be chemotactic for monocytes, T-lymphocytes, B-lymphocytes, dendritic cells, natural killer cells, eosinophils, or basophils but not neutrophils. The unique C chemokine, lymphotactin, was reported to be chemotactic for lymphocytes.[4] The sole C-X_3-C chemokine, fractalkine, a type 1 membrane protein containing a chemokine domain tethered on a long mucin-like stalk on endothelial cells, has been found to trigger the adhesion of T cells and monocytes.[5, 6] The recent discovery that HIV viruses use some chemokine receptors as co-receptors for entry into cells has sparked intense research activities on chemokines. The identification and kinetics of cells producing chemokines has value in the development of therapeutic interventions for chemokine-mediated pathologies.

References

1. Baggiolini, M. *et al.* (1997) Annu. Rev. Immunol. **15**:675
2. Schall, T.J. (1994) The Cytokine Handbook, 2nd ed. Thomson, A. ed., Academic Press, New York, 418.
3. Mackay, C.R. (1997) Current Biology **7**:R384
4. Kelnev, G.S. *et al.* (1994) Science **266**:1395.
5. Bazan, J.F. *et al.* (1997) Nature **385**:640.
6. Pan, Y. *et al.* (1997) Nature **387**:611.

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 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 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 x 10^6 cells/mL) with 1 μg/mL of LPS for 24 hours in the presence of 3 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.