Intracellular Staining Reagents

MIP-1β-Fluorescein Monoclonal Antibody

Catalog Number: IC271F

Lot Number: LIW05

100 Tests

Reagents Information

Carboxyfluorescein (CFS)-conjugated anti-MIP-1β: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone: 24006

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

Specificity: rhMIP-1β

Ig class: mouse IgG_{2B}

Intended Use

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

Principle of the Test

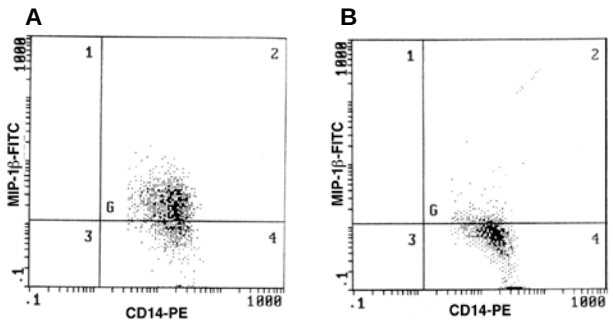
Fixed cells are permeabilized, allowing conjugated antibodies access to proteins within the cell. Cells are initially subjected to a fixation step in order to minimize leakage of proteins out of the cell. The conjugated antibody is allowed to penetrate and bind to its target cytokine within the cell. Following a final wash, the cells are analyzed on a flow cytometer. Flow cytometric analysis of CFS conjugates will generate a signal which can be detected in the FITC signal detector (usually FL1) while R-PE conjugates will generate signal which can be monitored by the detector reserved for phycoerythrin emission (usually FL2).

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. Make sure that all solids are 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-MIP-1β staining of LPS stimulated monocytes. A) CD14 gated monocyted stained with IC271F. B) Inhibition of IC271F staining by the addition of excess MIP-1β.



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, CD14, etc., in addition to the intracellular staining properties of the cells, we recommend that the investigator determines whether the surface protein is adversely affected by the fixation and permeabilization steps. Should this be the case, surface staining of cells prior to fixation and permeabilization is recommended.

Sample Staining

1. Harvest cells, and wash once in cold HBSS or PBS by spinning at 200 x g for 7 minutes.
2. If cells are to be surface stained with another monoclonal, follow the staining procedure indicated by the manufacturer.
3. Up to 1 x 10^6 cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Cells should be intermittently vortexed in order to maintain a single cell suspension. Following the fixation, cells are washed once in HBSS or PBS by again 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 about 200 μL of SAP buffer is left in the tube.
8. Cells are gently resuspended in the SAP buffer and 10 μL (or any 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. Resuspend the cells in each tube with 200 - 400 μL of PBS for final flow cytometric analysis.

For intracellular cytokine staining, the cells must first be fixed and permeabilized. The use of 4% paraformaldehyde in PBS as a fixative has given optimal results. Although other tissue fixative or formulations are currently available, we do not know how they may affect the staining properties of the monoclonals. For permeabilization, 0.1% saponin in a balanced salt solution has been found to be effective in allowing 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.

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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 producting 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 is known not to stain human cells, should be run to set quadrant statistics. For most antibodies, the binding of the antibody can be blocked with a preincubation of excess cytokine (10X molar excess should give >90% inhibition of staining). In some cases enhanced staining has been observed when attempting a cytokine block. This has been interpreted as being the result of the cytokine binding to its cell surface receptors and then being detected by the labeled monoclonal reagent. 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.

Production of cytokine 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 monocuclear cells (5 x10^6 cells/mL) with 500 ng/mL of LPS for 24 hours in the presence of 3 μM monensin.

The production level of a particular cytokine varies between different populations of cells as well as among cells of the same population. Indeed some cells may produce a cytokine at concentrations below the detection limits of the conjugated antibody. The investigator is encouraged to determine optimal titers with 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, has been helpful in enhancing detection of cytokines in low level secreting cells. To distinguish between detection of cell surface bound versus intracellular cytokines, we recommend treating the test cells with the unconjugated form of the same monoclonal being used for intracellular staining purposes.

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 in such a manner that renders it unrecognizable to the antibody. Strict attention to the fixation conditions must be followed in order to generate consistent results - 4% paraformaldehyde in PBS, for 10 minutes at room temperature has been found to be adequate.

Surface staining for cell determinants may be desired to identify cell populations. For some antibodies to CD markers, it may be necessary to stain the cells for surface antigen prior to fixation of the cells.

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.