Intracellular Staining Reagents

Anti-human TNFα-Phycoerythrin Monoclonal Antibody

Catalog Number: IC210P

Lot Number: LIH03

100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated anti-human TNFα: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 6402

Storage: 2° - 8° C in the dark

Ig class: mouse IgG_1

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.

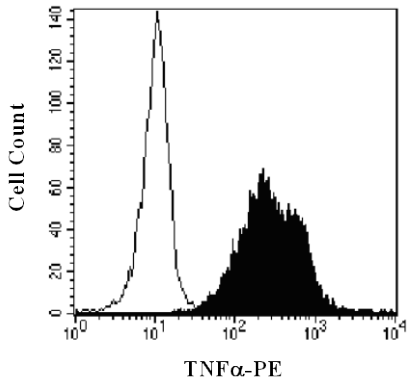
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN_3 in Hanks' Balanced Salt Solution (HBSS). Store at 18° - 24° C for no longer than 1 month.

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 fluorescein conjugates will generate a signal which can be detected in the FITC signal detector (usually FL1) while R-PE conjugates will generate a signal that can be monitored by the detector reserved for phycoerythrin emission (usually FL2).



Intracellular staining of PBMC+PMA+Calcium Ionomycin with PE-conjugated anti-human TNFα (Catalog # IC210P, filled histogram) or isotype control (Catalog # IC002P, open histogram).

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. 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. 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.

R&D Systems Inc.
1-800-343-7475

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 highly cellular (macrophage/cytotoxic T cell) response,[3 - 5] to a predominately humoral or antibody-mediated immunity.[6] IL-2 and 12, IFN-γ and TNF-β are associated with cellular immunity,[7 - 9] while interleukins 4, 5, 6, and 10 tend to accompany antibody responses.[10 - 12] By convention, cytokines promoting a cell-dominated response, like IL-2, IL-12, IFN-γ and TNF-β, are now known as type 1 cytokines; conversely cytokines promoting a humoral response, such as 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

1. Petrov, R. (1992) Immune Response Regulation. in *Encyclopedia of Immunology*, I.M. Roitt & P.J. Delves Ed., Academic Press Ltd. San Diego, CA.
2. Kaufmann, S.H.E. (1995) Immunol. Today **16**:338.
3. Fitch, F.W. (1992) Cell-Mediated Immunity. in *Encyclopedia of Immunology*, I.M. Roitt & P.J. Delves Ed., Academic Press Ltd. San Diego, CA.
4. Celada, A. & C. Nathan (1994) Immunol. Today **15**:100.
5. Apasov, S. *et al*. (1993) Curr. Opin. Immunol. **5**:404.
6. Berek, C. (1992) Humoral Immunity. in *Encyclopedia of Immunology*, I.M. Roitt & P.J. Delves Ed., Academic Press Ltd. San Diego, CA.
7. Waldmann, T. (1993) Immunol. Today **14**:264.
8. Trinchieri, G. (1995) Annu. Rev. Immunol. **13**:251.
9. Farrar, M.A. & R.D. Schreiber (1993) Annu. Rev. Immunol. **11**:571.
10. Spits, H. (1992) IL-4: Structure and function. CRC Press, Inc., Boca Raton, FL.
11. Takatsu, K. *et al*. (1994) Adv. Immunol. **57**:145.
12. Moore, K.W. *et al*. (1993) Annu. Rev. Immunol. **11**:165.
13. Mosmann, T.R. & R.L. Coffman (1989) Annu. Rev. Immunol. **7**:145.
14. Kelso, A. (1995) Immunol. Today **16**:374.
15. Prussin, C. & D.D. Metcalfe (1995) J. Immunol. Methods **188**:117.
16. Elson, L.H. *et al*. (1995) J. Immunol. **154**:4294.
17. Le Gros, G. & F. Erard (1994) Curr. Opin. Immunol. **6**:453.
18. Sad, S. *et al*. (1995) Immunity **2**:271.

Technical Note

Appropriate negative controls should be run to verify specificity and rule out background staining. An irrelevant antibody of the same isotype and concentration should be run to set quadrant statistics. Antibody binding can also be blocked with a preincubation of excess protein (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 protein that has bound to its cell surface receptor. 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.

The investigator is advised to determine whether activation strategies and/or a kinetics analysis may yield optimal results. The production level of a particular protein varies between different populations of cells as well as among cells within the same population. Indeed some cells may produce a protein 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 some proteins 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 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.