Intracellular Staining Reagents

Anti-human TNF-β/TNFSF1-Phycoerythrin Monoclonal Antibody

Catalog Number: IC2111P
Lot Number: KFB04
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated anti-human TNF-β: Supplied as 50 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 5808

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

Specificity: rhTNF-β

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.

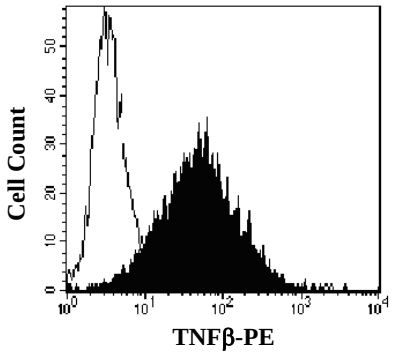
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN_3 in Hanks' Balanced Salt Solution (HBSS). Store at 18° - 24° C for no longer than 1 month.

Intended Use

The reagent is designed for flow cytometric applications intended to identify and quantitate cells possessing cytoplasmic forms of the 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 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 the human lymphoma cell line HuT 78 cells with PE-conjugated anti-human TNF-β (Catalog # IC2111P, filled histogram) or with isotype control (Catalog # IC002PF, 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 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.

For intracellular cytokine 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 facillitating 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

The presence or absence of distinct cytokines determines the quality and quantity of the immune response following antigenic challenge. The ability to monitor the intracellular and/or membrane associated presence of specific molecules has gained much research interest. Intracellular cytokine detection gained prominence in the elucidation of the $CD4^+$ Th1/Th2 T cell differentiation pathways. This intracellular staining technique has been extended to include many other molecules such as nuclear factors, enzymes, proteins modified by enzymatic activity and signal transducing molecules. As a result, the techniques that may yield optimal staining may require some modifications depending on both the cell type being assessed and the molecule of interest. In some cases, protein expression may be limited to the intracellular compartment, such as many of the interleukins, while molecules such as matrix metalloproteinases (MMPs) may be expressed intracellularly as well as on cell membrane surfaces where they may be subject to being cleaved and released as soluble entities. In some cases, secreted proteins may associate with accessory molecules such as receptor chains, inhibitors, and/or chaperone proteins that may assist in the secretion of the protein being investigated. In cases where the cells being assessed for the production of a specific cytokine also express receptors for the same cytokine, investigators need to be mindful of the potential of detecting intracellular as well as receptor bound cytokines during the "intracellular" staining process. These and other biologically relevant phenomena, such as non-mutually exclusive synthesis of certain molecules by cells of a specific phenotype, need to be taken into account for accurate interpretation of the staining results.

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 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 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.

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. The production level of a particular cytokine varies between different populations of cells as well as among cells within 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.

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.