Intracellular Staining Reagents

Anti-human IL-24-Phycoerythrin Monoclonal Antibody
Catalog Number: IC19653P
Lot Number: LTV02
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated anti-human IL-24: Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone: 283126

Ig class: mouse IgG_{2B}

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

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.

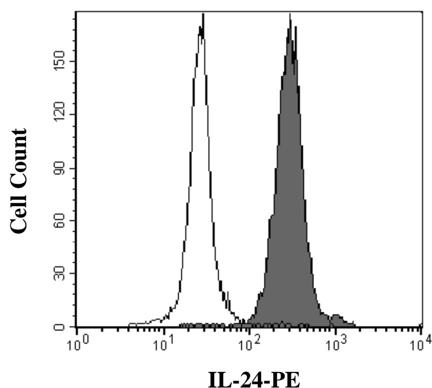
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN_3 in Hanks' Balanced Salt Solution (HBSS). Store at room temperature for no longer than 1 month.

Intended Use

The reagent is designed for flow cytometric applications intended to identify and quantify cells expressing 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 PE conjugates will generate a signal, which can be detected using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector, optimized to collect peak emissions at 565 - 605 nm.



Intracellular staining of human PBMC with PE-conjugated anti-human IL-24 (Catalog # IC19653P, filled histogram) or isotype control (Catalog # IC0041P, open histogram).

2008/02/27

Sample Preparation

Intracellular staining antibodies are designed for multiparameter flow cytometric analysis of cells. If it is desirable to stain for surface proteins (*e.g.* CD3, CD4, CD8), 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. If so, 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. 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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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1-800-343-7475

Background Information

Interleukin 24 (IL-24), also known as mda-7 (melanoma differentiation associated gene-7), is a newly discovered member of the IL-10 family of helical cytokines. The IL-24 gene encodes a precursor protein of 207 amino acids (aa) that contains a 48 aa signal sequence and an 18 kDa, 158 aa mature segment. There are three potential N-linked glycosylation sites, at least one of which is used. When secreted, IL-24 is a 35 - 40 kDa phosphorylated glycoprotein that apparently can exist as either a monomer or dimer. It is suggested that glycosylation is essential for activity. Mature human IL-24 shares 69% aa sequence identity with mouse and rat IL-24. Human IL-24 is active in rodent systems. Cells known to express IL-24 include B cells, $CD4^+$ T cells, NK cells, lymph node dendritic cells, monocytes, melanocytes, and melanoma cells. Functionally, IL-24 has diverse activities. At low concentrations on monocytes, it induces type I proinflammatory cytokines such as IFN-γ, IL-1β, IL-12 and TNF-α. At high concentrations, it is a strong inducer of apoptosis in tumor cells, but not in normal cells. IL-24 also has anti-angiogenic properties. It binds directly to IL-24 receptors on endothelial cells, activating STAT3 and blocking their differentiation. IL-24 binds and signals through two heterodimeric receptor complexes. One complex is the combination of IL-20 Rα and IL-20 Rβ, which is shared with IL-19 and IL-20. The second complex is a combination of IL-22 R and IL-20 Rβ, which is shared with IL-20.

References

1. Jiang, H. *et al*., 1995, Oncogene **11**:2477.
2. Jiang, H. *et al*., 1996, Proc. Natl. Acad. Sci. USA **93**:9160.
3. Wang, M. *et al*., 2002, J. Biol Chem. **227**:7341.
4. Chada, S. *et al*., 2004, Int. Immunopharmacol. **4**:649.
5. Pestka, S. *et al*., 2004, Annu. Rev. Immunol. **22**:929.
6. Chen, J. *et al*., 2003, Molec. Ther. **8**:220.

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 aid in setting quadrant statistics. Antibody binding can also be blocked with a pre-incubation of excess target 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 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.

Production of certain proteins varies between different populations of cells as well as among cells within the same population. Some cells may produce a protein 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, will enhance detection of the target protein 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 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.