Intracellular Staining Reagents

Anti-human IL-27-Fluorescein Monoclonal Antibody

Catalog Number: IC25261F

Lot Number: AATA01

100 Tests

Reagent Information

Carboxyfluorescein (CFS)-conjugated anti-human IL-27: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone: 307426

Ig class: mouse IgG_{2A}

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.

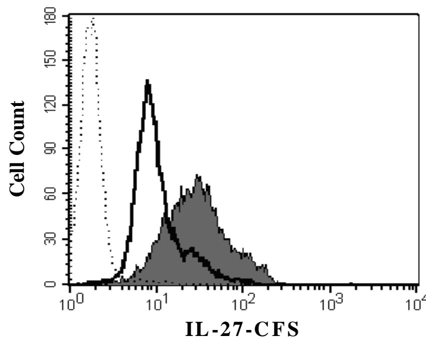
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN_3 in Hanks' Balanced Salt Solution (HBSS). Store at room temperature for no longer than 1 month.

Intended Use

The reagent is designed for flow cytometry applications intended to identify and quantitate 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 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 within the cell. Following a final wash, cells are analyzed on a flow cytometer. Analysis of fluorescein conjugates by flow cytometry 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 515 - 545 nm.



Intracellular staining of unstimulated human PBMC (open histogram-solid line) or PMA and rhIL-2 stimulated PBMC (filled histogram) with CFS-conjugated anti-human IL-27 (Catalog # IC25261F) or with isotype control antibody (Catalog # IC003F, open histogram-dotted line).

Sample Preparation

Intracellular staining antibodies are designed for analysis of cells by multiparameter flow cytometry. To stain for surface proteins (*e.g.* CD3, CD4, CD8) in addition to the intracellular protein, 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, 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 (i.e. both the staining and washing steps).

Sample Staining

1. Harvest cells, and wash twice in cold HBSS or PBS by centrifugation 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. 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 room temperature for 10 minutes.
4. Vortex the cells intermittently in order to maintain a single cell suspension. After fixation, wash the cells twice in HBSS or PBS followed by centrifugation at 200 x g for 7 minutes.
5. Harvest the cell pellet from each tube and resuspend in 2 mL of SAP buffer.
6. Centrifuge the cells at 200 x g for 7 minutes.
7. Decant the supernatant, 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 incubate for 30 - 45 minutes at room temperature **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 analysis by flow cytometry.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

IL-27 is a heterodimeric group 2 receptor ligand molecule that belongs to the IL-6/IL-12 family of long type I cytokines.[1] It is composed of EBI3 (EBV-induced gene 3), a 34 kDa glycoprotein that is related to the p40 subunit of IL-12 and IL-23, and p28, the recently cloned 28 kDa glycoprotein that is related to the p35 chain of IL-12.[2-4] The human EBI3 gene encodes a 229 amino acid (aa) precursor that contains a 20 aa signal peptide and 209 aa mature protein.[5] The mature region contains two potential N-linked glycosylation sites, two fibronectin type III domains, and two pairs of conserved cysteine residues with a WSxWS-like motif that places the molecule in the hematopoietin receptor family.[5] Although p40, the EBI3 counterpart in IL-12, is known to form homodimers, there is no evidence to date that EBI3 also homodimerizes. The amino acid sequence of human EBI3 is 61% identical to that of mouse EBI3. The human p28 gene encodes a 243 aa precursor that contains a 28 aa signal sequence and 215 aa mature region.[6] The mature region is characterized by the presence of four α-helices, placing it in the IL-6 family of helical cytokines. The amino acid sequence of human p28 is 74% identical to mouse p28. IL-27 is expressed by monocytes, endothelial cells and dendritic cells.[7] IL-27 binds to and signals through a heterodimeric receptor complex composed of WSX-1 (TCCR) and gp130. Evidence suggests IL-27 interacts only with WSX-1.[6, 8, 9] IL-27 has both anti- and pro-inflammatory properties. As an anti-inflammatory, IL-27 seems to induce a general negative feedback program that limits T and NK-T cell activity.[3, 7] At the onset of infection, IL-27 induces an IL-12 receptor on naïve $CD4^+$ T cells, making them susceptible to subsequent IL-12 activity (and possible Th1 development).[10]

References

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5. Devergne, O. *et al*. (1996) J. Virology **70**:1143.
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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.