

Reagent Information

Phycoerythrin (PE)-conjugated anti-mouse IL-21: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone: 149204

Ig class: rat 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.

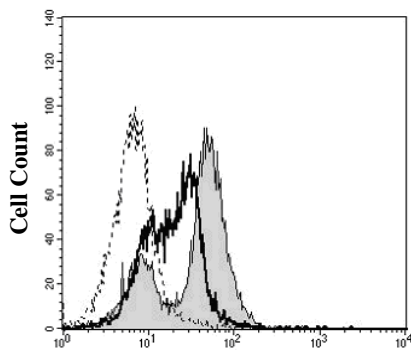
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN₃ in Hanks' Balanced Salt Solution (HBSS). Store at room temperature for no longer than 1 month.

Intended Use

The reagent is designed for flow cytometry applications intended to identify and quantitate cells 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. Analysis of PE 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 565 - 605 nm.



IL-21-PE

Intracellular staining on mouse splenocytes unactivated (open histogram, solid line) or activated with PMA and Calcium Ionomycin (filled histogram) using PE-conjugated anti-mouse IL-21 (Catalog # IC594P). Activated splenocytes were also stained with isotype control antibody (Catalog # J6019P, open histogram, dashed line).

Sample Preparation

Intracellular staining antibodies are designed for analysis of cells by multiparameter flow cytometry. If it is desirable 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. 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 centrifugation 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 10⁵ 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 intermittently in order to maintain a single cell suspension. Following 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 it 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 analysis by flow cytometry.

Background Information

The recently identified interleukin-21 (IL-21) and its receptor appear to play important roles in the regulation of the immune system. IL-21 is most related to IL-2, IL-4 and IL-15. IL-21 R, also called NILR (novel interleukin receptor), is a type I cytokine receptor with four conserved cysteine residues and an extracellular WSXWS motif. It is most closely related to IL-2 R β and IL-4 R α . Mouse IL-21 is a 146 amino acid (aa) residue protein with a 24 aa signal peptide. Mouse and human IL-21 share 57% amino acid identity. IL-21 is expressed by activated T cells. Although not fully elucidated, the IL-2 R γ (γ_c) chain appears to play a role in IL-21 R signaling. The IL-21/IL-21 R interaction appear to play important roles in B and T cell proliferation after antigen stimulation and NK cell maturation.

References

1. Parrish-Novak, *et al.*, 2000, Nature **408**:57.
2. Ozaki, K., *et al.*, 2000, Proc. Natl. Acad. Sci. USA **97**:11439.
3. Dumoutier, L. *et al.*, 2000, Proc. Natl. Acad. Sci. USA **97**:10144.
4. Asao, H. *et al.*, 2001, J. Immunol. **167**:1.

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.