Intracellular Staining Reagents

Anti-human STAT5a-Fluorescein Monoclonal Antibody

Catalog Number: IC21741F

Lot Number: AAJA02

100 Tests

Reagent Information

Carboxyfluorescein (CFS)-conjugated anti-human STAT5a: Supplied as 50 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone: 251610

Ig class: mouse IgG_1

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.

Methanol

PBS (Dulbecco's PBS)

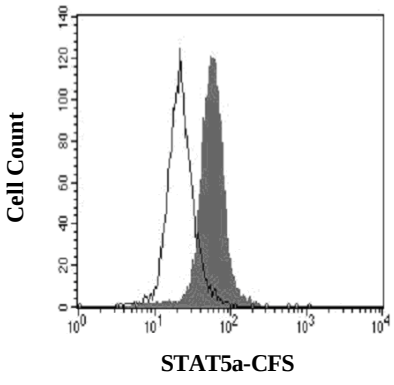
Bovine Serum Albumin (BSA)

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 Jurkat cells with CFS-conjugated anti-human STAT5a (Catalog # IC21741F, filled histogram) or with isotype control antibody (Catalog # IC002F, open histogram).

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.

Sample Staining

1. Harvest cells, and wash twice in 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. Following fixation, wash the cells twice in an isotonic PBS buffer supplemented with 0.5% BSA followed by centrifugation at 200 x g for 7 minutes.
5. While vortexing, resuspend the cells in 900 μL ice-cold (-20° C) methanol and incubate cells for 30 minutes at 4° C.
6. Centrifuge the cells at 200 x g for 7 minutes.
7. Decant the supernatant. Wash twice in PBS/BSA buffer. After the second wash ensure that approximately 200 μL of buffer remains in the tube.
8. Gently resuspend the cells in the remaining PBS/BSA 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 PBS/BSA 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.

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

Background Information

Signal Transducer and Activator of Transcription 5a (STAT5a) is a member of the STAT family of proteins that mediate cytokine signaling by acting as signal transducers in the cytoplasm and transcriptional activators in the nucleus. STAT5a and STAT5b are encoded by separate genes and share 93% amino acid identity.

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.