Intracellular Staining Reagents

Anti-mouse IL-17D-Allophycocyanin Monoclonal Antibody

Catalog Number: IC2274A
Lot Number: AAHY02
100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated anti-mouse IL-17D: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone: 312724

Ig class: rat 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.

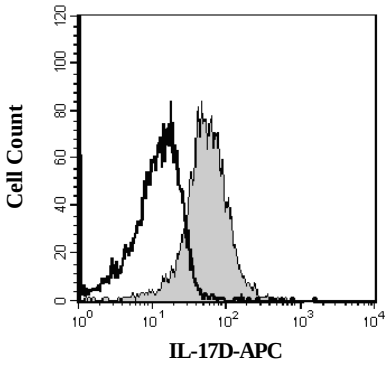
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN_3 in Hanks' Balanced Salt Solution (HBSS). Store at room temperature for no longer than 1 month.

Intended Use

The reagent is designed for flow cytometric 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. Flow cytometric analysis of APC conjugates will generate a signal which can be detected using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



Intracellular staining of mouse splenocytes with APC-conjugated anti-mouse IL-17D (Catalog # IC2274A, filled histogram) or with isotype control antibody (Catalog # IC006A, open histogram).

Sample Preparation

Intracellular staining antibodies are designed for multiparameter flow cytometric analysis of cells. 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 spinning 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 HBSS or PBS by centrifuging 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 final flow cytometric analysis.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

The Interleukin 17 (IL-17) family proteins, comprising six members (IL-17, IL-17B through IL-17F), are secreted, structurally related proteins that share a conserved cystine-knot fold near the C-terminus, but have considerable sequence divergence at the N-terminus.[1, 2, 6] With the exception of IL-17B, which exists as a non-covalently linked dimer, all IL-17 family members are disulfide-linked dimers.[3] IL-17 family proteins are pro-inflammatory cytokines that induce local cytokine production and are involved in the regulation of immune functions.[1, 2, 6] Two receptors (IL-17 R, and IL-17B R), which are activated by IL-17 family members, have been identified. In addition, at least three additional orphan type I transmembrane receptors with homology to IL-17 R, including IL-17 RL (IL-17 RC), IL-17 RD, and IL-17 RE, have also been reported,[1 - 6] Mouse IL-17D is synthesized as a 205 amino acid (aa) precursor that contains a putative aa signal peptide and a 181 aa mature segment. The mature region contains two potential N-linked glycosylation sites and eight cysteines, four of which are involved in the formation of a modified cysteine-knot motif.[5] The molecule is reported to exist as a 53 kDa disulfide-linked homodimer.[2, 5] Given that its predicted homodimeric molecular weight is 40 kDa, the molecule is presumably glycosylated. In the mature region, mouse IL-17D is 88% aa identical to human IL-17D. There is less that 30% aa identity between mouse IL-17D and other members of the mouse IL-17 family. IL-17D is expressed in skeletal muscle, adipose tissue, fetal liver, and heart, plus resting $CD4^+$ T cells and $CD19^+$ B cells.[1] R&D Systems has shown IL-17D binding to a mouse IL-17 R/Fc construct in a functional ELISA. IL-17D is known to induce the production of IL-8, IL-6 and GM-CSF.[5]

References

1. Aggarwal, S. and A.L. Gurney, 2002, J. Leukoc. Biol. **71**:1 - 8.
2. Moseley, T.A. *et al*., 2003 Cytokine & Growth Factor Rev. **14**:155 - 174.
3. Hymowitz, S.G. et al., 2001, EMBO J. **20**:5332 - 5341.
4. Haudenschild, D. *et al*., 2002, J. Biol. Chem. **277**:4309 - 4316.
5. Starnes, T. *et al*., 2002, J. Immunol. **169**:642.
6. Kolls, J.K. and A. Linden, 2004, Immunity **21**:467.

Technical Notes

Appropriate negative controls should be used to verify specificity and rule out background staining. An irrelevant antibody of the same isotype and concentration should be tested 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 to 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.