Monoclonal Anti-human/mouse IL-12/IL-35 p35-APC

Catalog Number: IC2191A
Lot Number: ABAR01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human/mouse IL-12/IL-35 p35: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 27537

Isotype: mouse IgG_1

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

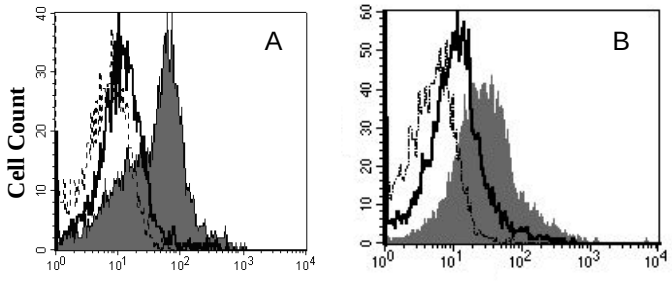
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing IL-12/IL-35 p35 within a population and qualitatively determine the density of intracellular IL-12/IL-35 p35 by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *Sf* 21-derived, recombinant human IL-12 p35 subunit (rhIL-12 p35; aa 23 - 219; Accession # P29459). The IgG fraction of the ascites fluid was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of IL-12/IL-35 p35 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



IL-12/IL-35 p35-APC

(A) Human PBMC, resting (open histogram-solid line) or LPS-treated (filled histogram), and (B) mouse splenocytes, resting (open histogram-solid line) or LPS-treated (filled histogram) were stained with APC-conjugated anti-human/mouse IL-12/IL-35 p35 (Catalog # IC2191A) or APC-conjugated isotype control (Catalog # IC002A, open histograms-dotted line).

Background Information

Interleukin 12 (IL-12) and interleukin 35 (IL-35) are heterodimeric cytokines composed of α and β chains. IL-12 is composed of p35 and p40 subunits, while IL-35 is comprised of p35 paired with EBI-3.[1] In mice, IL-35 is produced by $FoxP3^+$ regulatory T cells and may function as an inhibitory cytokine to suppress T cell proliferation.[2] Human $FoxP3^+$ Tregs do not constitutively express IL-35,[3] but expression may be induced by activated dendritic cells.[4]

References

1. Collison, L.W. and D.A.A. Vignali (2008) Immunol. Rev. **226**:248.
2. Collison, L.W. *et al*. (2007) Nature **450**:566.
3. Bardel, E. *et al.* (2008) J. Immunol. **181**: 6898.
4. Seyerl, M. *et al.* (2010) Eur. J. Immunol. **40**: 321.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see Reagents Not Provided).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Resuspend up to 1 x 10^6 cells in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubate at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 μL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_1 antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine optimal reagent amount for their specific use.

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.

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