

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human/mouse IL-12 R β 2: Supplied as 25 μ g of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 305719

Isotype: mouse IgG₁

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

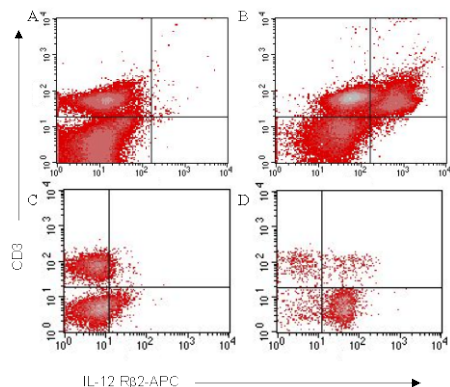
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing IL-12 R β 2 within a population and qualitatively determine the density of IL-12 R β 2 on cell surfaces 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, NS0-derived, recombinant human Interleukin 12 Receptor beta 2 extracellular domain (rhIL-12 R β 2; aa 24 - 622; Accession # Q99665). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of IL-12 R β 2 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.



Human PBMCs, resting (panel A) or Th1-differentiated (panel B), and mouse splenocytes, resting (panel C) or Th1-differentiated (panel D), were stained with APC-conjugated anti-human/mouse IL-12 R β 2 (Catalog # FAB1959A) and anti-human or anti-mouse CD3. The quadrant markers were set based on isotype control staining (Catalog # IC002A).

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

Background Information

The biological activities of IL-12 are mediated through a high-affinity receptor complex composed of the IL-12 receptor β 1 (IL-12 R β 1) and IL-12 receptor β 2 (IL-12 R β 2) subunits. IL-12 R β 2 is a 130 kDa protein that interacts with p35 and is associated with Jak2. The expression of IL-12 R β 2 is absent on naïve T cells. Activation of T cells via TCR up-regulates IL-12 R β 2 expression on human Th1, but not Th2 cells.¹⁻⁴

References

- Trinchieri, G. *et al.* (2003) *Immunity* **19**:641.
- Brombacher, F. *et al.* (2003) *Trends Immunol.* **23**:207.
- Trinchieri, G. (2003) *Nat. Rev. Immunol.* **3**:133.
- Rogge, L. *et al.* (1997) *J. Exp. Med.* **185**:825.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using human Th1-differentiated PBMCs (Shaojing, C. *et al.* (2007) *Nat. Immunol* **8**:723.). Briefly, PBMCs were treated with rhIL-12 (5 ng/mL) and anti-human IL-4 (10 μ g/mL) for 2 days, then activated with PMA (50 ng/mL) plus ionomycin (200 ng/mL) for 3 hours.

- Cells may be Fc-blocked with 1 μ g of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 μ L of conjugated antibody was added to up to 1 x 10⁶ cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
- The cells were resuspended in Flow Cytometry Staining Buffer for analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG₁ antibody. This procedure may need to be modified, depending upon 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.