Monoclonal Anti-mouse IL-27-CFS

Catalog Number: IC1834F
Lot Number: ABBM01
100 Tests

Reagents Provided

Carboxyfluorescein (CFS)-conjugated rat monoclonal anti-mouse IL-27: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 234205

Isotype: rat IgG_{2B}

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

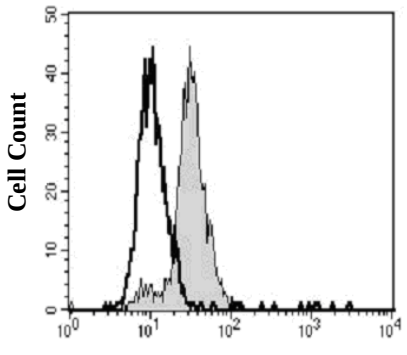
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing IL-27 within a population and qualitatively determine the density of intracellular IL-27 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 rat immunized with purified, CHO-derived, recombinant mouse IL-27 (rmIL-27; aa 29 - 234 of p28; Accession # Q8K3IC and aa 19 - 228 of EBI-3; Accession # O35228). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to a CFS fluorochrome. Intracellular expression of IL-27 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.



RAW264.7 cells, activated with LPS, were stained with CFS-conjugated anti-mouse IL-27 (Catalog # IC1834F, filled histogram) or isotype control (Catalog # IC013F, open histogram).

Background Information

Interleukin 27 (IL-27) is a novel heterodimeric cytokine comprised of the IL-12 p35-related protein, p28, and the IL-12 p40-related protein, EBI-3 (Epstein-Barr virus-induced gene 3). IL-27 binds TCCR/WSX-1 on naïve $CD4^+$ T cells and induces the expression of a functional IL-12 receptor, making these cells sensitive to IL-12-mediated Th1 cell development.

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. 5 x 10^5 cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, 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 cells were incubated for 30 minutes at room temperature **in the dark**.
6. Cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with CFS-labeled rat IgG_{2B} antibody. This procedure may need to be modified, depending on 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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