Polyclonal
Anti-human APRIL/TNFSF13-Phycoerythrin

Catalog Number: IC884P
Lot Number: ACEJ01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-human APRIL/TNFSF13: Supplied as 50 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: goat IgG

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

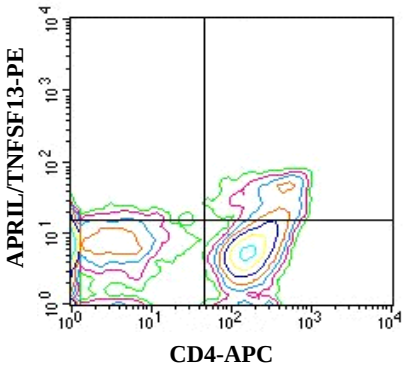
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing APRIL/TNFSF13 within a population and qualitatively determine the density of intracellular APRIL/TNFSF13 by flow cytometry.

Product Description

Produced in goats immunized with purified, *E. coli*-derived, recombinant human APRIL/TNFSF13 (amino acids 110 - 250). APRIL/TNFSF13 specific IgG was purified by human APRIL/TNFSF13 affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Intracellular expression of APRIL/TNFSF13 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.



Th2-stimulated PBMCs were stained with PE-conjugated anti-human APRIL/TNFSF13 (Catalog # IC884P) and APC-conjugated anti-human CD4 (Catalog # FAB3791A).

Background Information

APRIL (a proliferation inducing ligand), also known as TNFSF13, TALL2, and TRDL1, is a member of the TNF ligand superfamily. It is synthesized as a 32 kDa type II transmembrane protein, which is cleaved by furin in the Golgi to release a 17 kDa soluble molecule. Secreted APRIL consists almost entirely of a single TNF homology domain. Little or no transmembrane APRIL is expressed on the cell surface. Alternate splicing generates isoforms with short deletions at the N- or C-terminus. Both APRIL, and the closely related protein, BAFF, signal through the TNF superfamily receptors, TACI and BCMA, to promote cellular proliferation and protect against apoptosis. APRIL can form bioactive heterotrimers with BAFF. Human APRIL shares 85% amino acid sequence identity with mouse and rat APRIL.

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. Up to 1 x 10^6 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, 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 PE-labeled goat IgG antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine the 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.

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