Polyclonal
Anti-human ILT6/CD85e-Phycoerythrin

Catalog Number: IC2574P
Lot Number: ACKV01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated goat polyclonal anti-human ILT6/CD85e: Supplied as 10 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

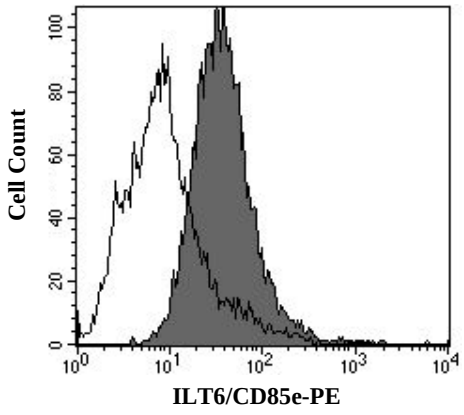
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing ILT6/CD85e within a population and qualitatively determine the density of intracellular ILT6/CD85e by flow cytometry.

Product Description

This antibody was produced in goats immunized with purified, NS0-derived recombinant human ILT6/CD85e (Accession # Q8N6C8) and was purified by human ILT6/CD85e affinity chromatography. The purified antibody was then conjugated to a PE fluorochrome. Intracellular expression of ILT6/CD85e 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.



PBMC monocytes were stained with PE-conjugated anti-human ILT6/CD85e (Catalog # IC2574P, filled histogram) or PE-conjugated control antibody (Catalog # IC108P, open histogram).

Background Information

ILT6, also known as CD85e, LIR4, and LILRA3, contains four Ig-like C2-type domains and is the only ILT family member that lacks a transmembrane and cytoplasmic domain. ILT proteins modulate immune responses through interactions with class I MHC molecules. Several polymorphisms of ILT6 have been described, and loss of ILT6 is associated with the development of multiple sclerosis. Human and chimpanzee ILT6 share 84% amino acid sequence identity. ILT6 orthologs have not been described in other species.

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 upon 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.

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