

Reagents Provided

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

Clone #: 27232

Isotype: mouse IgG₁

Reagents Not Provided

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

Storage

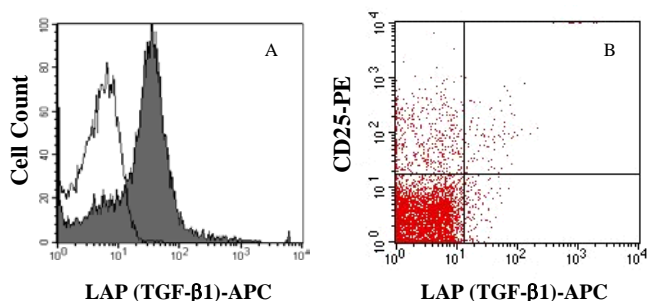
Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing LAP (TGF- β 1) within a population and qualitatively determine the density of LAP (TGF- β 1) 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 Sf 21-derived recombinant human latency associated peptide of TGF- β 1 (rhLAP (TGF- β 1); aa 30 - 278; Accession# P01137.2). 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 LAP (TGF- β 1) 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.



(A) Human platelets or (B) mouse splenocytes were stained with APC-conjugated anti-human/mouse LAP (TGF- β 1) (Catalog # FAB2463A, filled histogram) or isotype control (Catalog # IC002A, open histogram). Splenocytes were costained with PE-conjugated anti-mouse CD25 (Catalog # FAB2438P), and quadrant markers were set based upon isotype control staining.

Background Information

TGF- β is secreted by cells in the form of an inactive complex. This complex consists of TGF- β associated non-covalently with a protein designated the latency associated peptide (LAP). TGF- β and LAP represent components of a pro-peptide that is cleaved in a post-golgi compartment prior to secretion. LAP and TGF- β each consist of a disulfide-linked homodimer and the association of these two components renders TGF- β inactive and inaccessible to anti-TGF- β antibodies.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using human platelets and mouse splenocytes.

- Cells may be Fc-blocked with 1 μ g of human or mouse IgG/ 10^5 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 1×10^6 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 (Catalog# FC002) or Mouse (Catalog# FC003) Lyse Buffer.
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for 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.