Monoclonal Anti-human LAP (TGF-β1)-Alexa Fluor® 488

Catalog Number: FAB2463G
Lot Number: ABVC01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated mouse monoclonal anti-human LAP (TGF-β1): Supplied as 25 μg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 27232

Isotype: mouse IgG_1

Reagents Not Provided

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

Storage

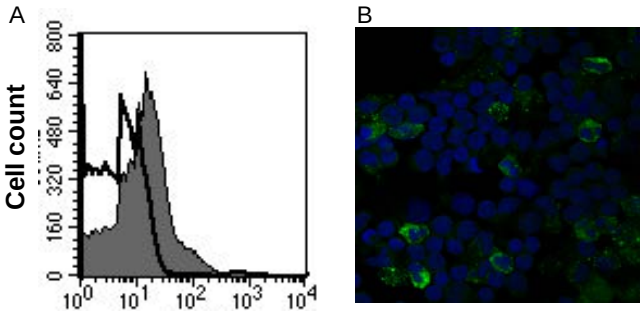
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing LAP (TGF-β1) within a population and qualitatively determine the density of LAP (TGF-β1) 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-β1 (rhLAP (TGF-β1); 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 Alexa Fluor® 488 fluorochrome. Cell surface expression of LAP (TGF-β1) 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.



LAP (TGF-β1)-Alexa Fluor® 488

A) Human platelets were isolated from whole blood, and stained with either Alexa Fluor® 488-conjugated isotype control (Catalog # IC002G) or anti-human LAP (TGF-β1) (Catalog # FAB2463G).

B) Alexa Fluor® 488-conjugated anti-human LAP (TGF-β1; Catalog # FAB2463G) was also used to detect LAP (TGF-β1) by ICC staining of human PBMCs stimulated overnight with CD3/CD28/TGF-β/IL-2 (green). Nuclei were counterstained with DAPI (blue).

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 PBMCs.

1. Cells may be Fc-blocked with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 5 μL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. 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).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 488-labeled mouse IgG_1 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.

Immunocytochemistry Validation

This antibody has been tested for immunocytochemistry at 5 μg/mL on human PBMCs stimulated overnight with CD3/CD28/TGF-β/IL-2 (induced regulatory T cells).

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.

Legal