Monoclonal Anti-human Latent TGF-β bp1-APC

Catalog Number: IC388A
Lot Number: ABHS02
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human Latent TGF-β bp1: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 35409

Isotype: mouse IgG_1

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

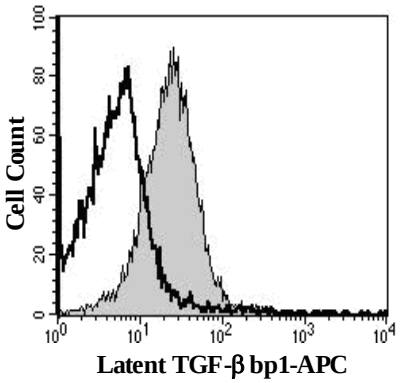
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing Latent TGF-β bp1 within a population and qualitatively determine the density of intracellular Latent TGF-β bp1 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 partially purified, large latent transforming growth factor beta 1 complex from human platelets. The IgG fraction of ascites fluid was purified by Protein A affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of Latent TGF-β bp1 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.



PC-3 cells were stained with APC-conjugated anti-human Latent TGF-β bp1 (Catalog # IC388A, filled histogram) or isotype control (Catalog # IC002A, open histogram).

Background Information

TGF-β is synthesized as high molecular weight latent complexes. In platelets, the large latent TGF-β1 complex is composed of three components: mature TGF-β1 dimer, latency associated peptide, and latent TGF-β binding protein (LTBP).[1-3] The cDNAs for four binding proteins (LTBP-1, -2, -3 and -4) have now been cloned.[4,5]

References

1. Miyazono, K. *et al.* (1991) EMBO J. **10:**1091.
2. Moren, A. *et al.* (1994) J. Biol. Chem. **269:**32469.
3. Yin, W. *et al.* (1995) J. Biol. Chem. **270:**10147.
4. Kanzaki, T. *et al.* (1990) Cell **61:**1051.
5. Saharinen, J. *et al.* (1998) J. Biol. Chem. **273:** 18459.

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 APC-labeled mouse IgG_1 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.

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