

Reagents Provided

Allophycocyanin (APC)-conjugated rat monoclonal anti-human TIM-3: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 344823

Isotype: rat IgG_{2A}

Reagents Not Provided

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

Storage

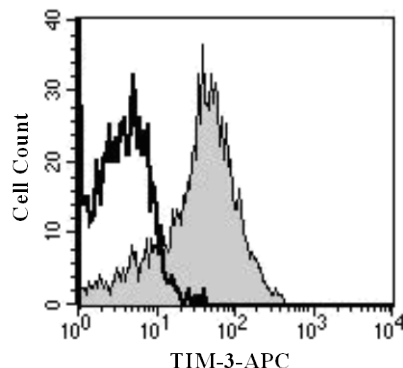
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing TIM-3 within a population and qualitatively determine the density of TIM-3 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 rat immunized with purified, NS0-derived, recombinant human TIM-3 extracellular domain (rhTIM-3; aa 22 - 200; Accession # NP_116171). 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 TIM-3 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.



Whole blood monocytes were stained with APC-conjugated anti-human TIM-3 (Catalog # FAB2365A, filled histogram) or APC-conjugated isotype control (Catalog # IC006A, open histogram).

Background Information

T cell immunoglobulin mucin-3 (TIM-3) is a member of the T cell Immunoglobulin- and Mucin-domain-containing family of type I membrane glycoproteins that regulate autoimmune and allergic disease. TIM-3 is selectively expressed on Th1 cells and interacts with galectin-9. It negatively regulates Th1 responses and affects macrophage activation. The 280 amino acid (aa) mature human TIM-3 contains a V-type Ig-like domain that shows multiple polymorphisms, followed by a mucin-like domain in the 171 aa extracellular region, which shares 60% aa identity with the mouse TIM-3 ECD. One splice variant is truncated within the mucin domain and presumably is secreted.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using whole blood monocytes.

- Cells may be Fc-blocked with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL of conjugated antibody was added to up to 1 x 10⁶ 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 Lyse Buffer (Catalog # FC002).
- 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 APC-labeled rat IgG_{2A} 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.