

Reagents Provided

Allophycocyanin (APC)-conjugated rat monoclonal anti-mouse TREM-1: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 174031

Isotype: rat IgG_{2A}

Reagents Not Provided

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

Storage

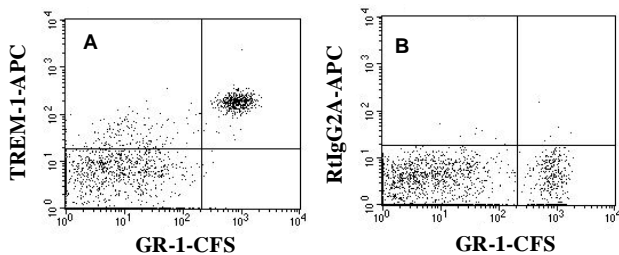
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing TREM-1 within a population and qualitatively determine the density of TREM-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 rat immunized with purified NS0-derived recombinant mouse TREM-1 (aa 21-202; Accession # Q9JKE2). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of TREM-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.



Mouse peripheral blood cells were stained with CFS-conjugated anti-mouse GR-1 (Catalog # FAB1037F) and A) APC-conjugated anti-mouse TREM-1 (Catalog # FAB1187A) or B) isotype control (Catalog # IC006A).

Background Information

TREM-1 (Triggering Receptor Expressed on Myeloid cells) is a type I transmembrane protein having a single Ig-like domain. It associates with the adapter protein, DAP12, to deliver an activating signal. Several other TREM family members have been reported that are structurally similar but share less than 30% amino acid identity. TREM-1 is expressed on blood neutrophils and a subset of monocytes, and expression is upregulated by bacterial LPS. Engagement of TREM-1 with a monoclonal antibody leads to expression of IL-8, MCP-1, and TNF-α suggesting that this receptor plays an important role in inflammatory responses. TREM-1 is expressed at high levels on neutrophils of patients with microbial sepsis and in mice with LPS-induced shock. Blockade of TREM-1 with a TREM-1/Fc fusion protein protected mice against LPS-induced shock.

References

- Bouchon, A. (2000) J. Immunol. **164**:4991.
- Bouchon, A. (2001) Nature **410**:1103.
- Nathan, C. and A. Ding (2001) Nature Med. **7**:530.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse peripheral blood cells.

- Cells may be Fc-blocked with 1 µg of mouse 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 Mouse Lyse Buffer (Catalog # FC003).
- 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.