

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse TLR6:

Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 418601

Isotype: rat IgG_{2A}

Reagents Not Provided

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

Storage

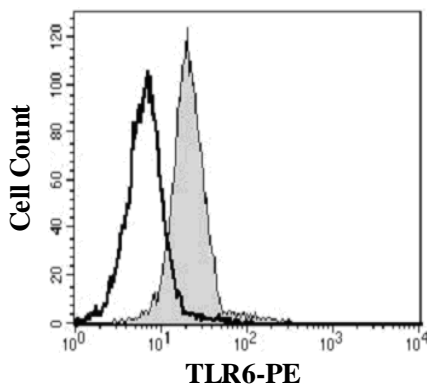
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing TLR6 within a population and qualitatively determine the density of TLR6 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 HEK293 cells transfected with mouse TLR6 (Accession # NP_035734). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of TLR6 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.



RAW267.7 cells were stained with PE-conjugated anti-mouse TLR6 (Catalog # FAB1533P, filled histogram) or isotype control (Catalog # IC006P, open histogram).

Background Information

TLR6 is a ~105 kDa cell surface type I transmembrane protein of the IL-1 receptor/Toll-like receptor (TLR) superfamily. TLRs are leucine-rich repeat-containing pattern recognition receptors for pathogen-associated molecular patterns that activate the innate immune system. TLR6 is expressed on macrophages, monocytes, neutrophils, and dermal endothelial cells in ligand-independent association with TLR2. The mouse TLR6 extracellular domain shares 72%, and 86% amino acid (aa) identity with human and rat TLR6, respectively, and 59% aa identity with mouse TLR1.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using RAW264.7 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 1 - 2.5 x 10⁵ 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 PE-labeled rat IgG_{2A} antibody. This procedure may need to be modified, depending upon 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.