

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human TLR7: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 533707

Isotype: mouse IgG_{2A}

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

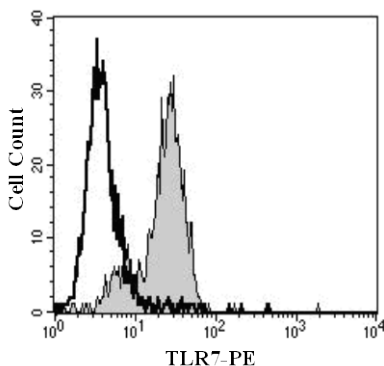
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing TLR7 within a population and qualitatively determine the density of intracellular TLR7 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 purified, *E. coli*-derived, recombinant human toll-like receptor 7 (rhTLR7; aa 360 - 516). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to a PE fluorochrome. Intracellular expression of TLR7 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.



Ramos were stained with PE-conjugated anti-human TLR7 (Catalog # IC5875P, filled histogram) or PE-conjugated isotype control (Catalog # IC003P, open histogram).

Background Information

Toll-like receptor 7 (TLR7) is a 120 kDa (predicted, unglycosylated) type I transmembrane glycoprotein and member of the Toll-like receptor family. Human TLR7 is synthesized as a 1049 amino acid (aa) precursor that contains a 26 aa signal sequence, an 803 aa extracellular domain (ECD), a 21 aa transmembrane sequence, and a 189 aa cytoplasmic domain. Mature human TLR7 is 81% aa identical to mature mouse TLR7. TLR7 is detected in brain, placenta, spleen, stomach, small intestine, lung, and in plasmacytoid pre-dendritic cells. Functionally, TLR7 participates in the innate immune response to microbial agents.

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 procedures.
3. Up to 1×10^6 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 PE-labeled mouse IgG_{2A} 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.