Polyclonal
Anti-human TLR1-Allophycocyanin

Catalog Number: FAB1484A
Lot Number: AANV01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated goat polyclonal anti-human TLR1: Supplied as 10 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Isotype: goat IgG

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

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

Intended Use

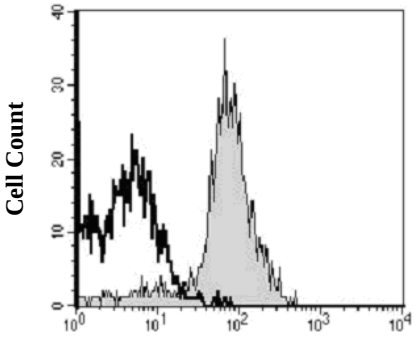
Designed to quantitatively determine the percentage of cells bearing TLR1 within a population and qualitatively determine the density of TLR1 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled polyclonal antibody, which binds to cells expressing TLR1. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing TLR1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of TLR1. Cell surface expression of TLR1 is determined by flow cytometry using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

Allophycocyanin-conjugated goat anti-human TLR1: Use as is; no preparation necessary.



Human monocytes were stained with APC-conjugated anti-human TLR1 (Catalog # FAB1484A, filled histogram) or isotype control (Catalog # IC108A, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells should then be transferred to a 5 mL tube for staining with the antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 μL of cells (1×10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells should be Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated TLR1 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted TLR1 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
6) Finally, resuspend the cells in 200 - 400 μL of PBS buffer for analysis by flow cytometry.
7) As a control for analysis, cells in a separate tube should be treated with APC-labeled goat IgG antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

TLR1 was cloned from a human erythroleukemic (TF-1) cell line-derived cDNA library (Accession # U88540) (1). TLR1 maps to chromosome 4p14 and its sequence encodes a putative 786 amino acid (aa) protein with 18 N-terminal LRRs and a calculated molecular weight of 84 kDa (1, 2). TLR1 is most closely related to TLR6 and TLR10 with 68% and 48% overall aa sequence identity, respectively.

In vivo, two different sized transcripts for TLR1 are observed suggesting that the mRNA is alternatively spliced to generate two different forms of the protein. TLR1 mRNA is ubiquitously expressed and found at higher levels than the other TLRs (1). Of the major leukocyte populations, TLR1 is most highly expressed by monocytes, but is also expressed by macrophages, dendritic cells (DCs), polymorphonuclear leukocytes, B, T, and NK cells (3 - 6). *In vitro*, TLR1 mRNA and protein expression is upregulated in monocytic leukemic (THP-1) cells upon PMA-induced differentiation. While TLR1 expression is most significantly upregulated by autocrine IL-6, it is also elevated by IFN-β, IL-10, and TNF-α. However, TLR1 level is unaffected by exposure to both Gram-positive and Gram-negative bacteria (3). *Ex vivo*, both monocyte and granulocyte TLR1 expression is down-regulated after exposure to Gram-negative bacteria (3). TLR1 forms a heterodimer with TLR2 (as do other TLRs). While the significance of TLR heterodimerization is not clearly understood, it is thought that some TLRs, such as TLR1, function to specify or enhance the PAMP sensitivity of TLR2 (7 - 9). However, there is evidence that TLR2 may not signal as a homodimer and that TLR1 in this case is required for downstream functionality (10). TLR1 also heterodimerizes with TLR4 to inhibit TLR4 activity (11).

References

1. Rock, F.L. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:588.
2. Dunne, A. and L.A.J. O'Neill (2003) Sci. STKE **2003**:re3.
3. Zarember, K.A. and P.J. Godowski (2002) J. Immunol. **168**:554.
4. Muzio, M. *et al.* (2000) J. Immunol. **164**:5998.
5. Hornung, V. *et al.* (2002) J. Immunol. **168**:4531.
6. Ochoa, M.T. *et al.* (2003) Immunology **108**:10.
7. Ozinsky, A. *et al.* (2000) Proc. Natl. Acad. Sci. USA **97**:13766.
8. Wyllie, D.H. *et al.* (2000) J. Immunol. **165**:7125.
9. Hajjar, A.M. *et al.* (2001) J. Immunol. **166**:15.
10. Zhang, H. *et al.* (2002) FEBS Lett. **532**:171.
11. Spitzer, J.H. *et al.* (2002) Eur. J. Immunol. **32**:1182.

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.