Polyclonal Anti-human TLR3-APC

Catalog Number: IC1487A
Lot Number: ACVX01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated goat polyclonal anti-human TLR3: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: goat IgG

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

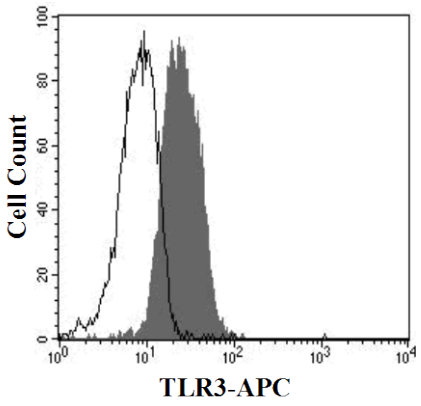
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells containing TLR3 within a population and qualitatively determine the density of intracellular TLR3 by flow cytometry.

Product Description

This antibody was produced in goats immunized with purified, NS0-derived, recombinant human Toll-Like Receptor 3 (rhTLR3; Accession # O15455). Human TLR3 specific IgG was purified by human TLR3 affinity chromatography. The purified antibody was then conjugated to an APC fluorochrome. Intracellular expression of TLR3 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.



A549 cells were stained with APC-conjugated anti-human TLR3 (Catalog # IC1487A; filled histogram) or APC-conjugated isotype control (Catalog # IC108A; open histogram).

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.

Background Information

Human TLR3 is a 116 kDa type I transmembrane glycoprotein that belongs to the mammalian Toll-Like Receptor family of pathogen pattern recognition molecules.[1] TLR3 is found in phagosomes, where the acidic pH enables binding of internalized double-stranded RNA and mRNA from viruses, parasites, and necrotic virally-infected cells.[2-6] Ligand binding by TLR3 induces receptor dimerization, leading to the release of inflammatory cytokines and dendritic cell maturation.[3, 4, 6, 8] TLR3 is expressed in dendritic cells, macrophages, microglia, and astrocytes, and is upregulated by IFN-β and LPS.[4, 8-10] TLR3 expression is also induced by lung fibroblasts and epithelial cells by respiratory syncytial virus infection.[7] The extracellular domain (ECD) of human TLR3 shares 80%, 79%, and 77% aa sequence identity with the ECD of rat, mouse, and bovine TLR3, respectively.

References

1. Sen, G.C. & S.N. Sarkar (2005) Cytokine Growth Factor Rev. **16**:1.
2. Nishiya, T. *et al*. (2002) J. Biol. Chem. **280**:37107.
3. de Bouteiller, O. *et al*. (2005) J. Biol. Chem. **280**:38133.
4. Alexopoulou, L. *et al*. (2001) Nature **413**:732.
5. Aksoy, E. *et al*. (2005) J. Biol. Chem. **280**:277.
6. Kariko, K. *et al*. (2004) J. Biol. Chem. **279**:12542.
7. Rudd, B.D. *et al*. (2005) J. Virol. **79**:3350.
8. Farina, C. *et al*. (2005) J. Neuroimmunol. **159**:12.
9. Heinz, S. *et al*. (2003) J. Biol. Chem. **278**:21502.
10. Wang, T. *et al*. (2004) Nat. Med. **10**:1366.

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 procedure.
3. Resuspend up to $1x10^6$ cells in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubate 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 analysis, cells in a separate tube should be treated with APC-labeled goat IgG 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.