Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human Calreticulin: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 326203
Isotype: mouse IgG₂B

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 Calreticulin within a population and qualitatively determine the density of Calreticulin 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 mouse immunized with purified, E. coli-derived, recombinant mouse Calreticulin (Accession # P27797) extracellular domain. 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 Calreticulin 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.

Whole blood granulocytes were stained with APC-conjugated anti-human Calreticulin (Catalog # FAB3898A, filled histogram) or APC-conjugated isotype control (Catalog # IC0041A, open histogram).

Background Information

Human Calreticulin is a 55 - 60 kDa, 400 amino acid (aa), variably glycosylated, intracellular and extracellular Ca²⁺-binding lectin that is ubiquitously expressed. It consists of three domains: a 180 aa N-terminal globular region, a 111 aa proline rich or P domain, and a 109 aa C-terminus. The 180 aa N-terminus (aa 18 - 197) is termed Vasostatin. It is unclear if it is ever generated naturally via proteolytic processing. The Vasostatin domain has many functions. It binds to RNA (aa 18 - 27), has autocatalytic phosphorylase activity (aa 77 - 197), binds to a KxFFKR motif on steroid hormone receptors, and serves as a lectin-type chaperone for molecules localized to the endoplasmic reticulum. It also shows anti-angiogenic activity, presumably by binding to laminin carbohydrates and blocking endothelial cell adhesion and proliferation. Human Calreticulin is 94% identical to mouse and rat Calreticulin.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using whole blood granulocytes.

1. Cells may be Fc-blocked with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 10 µL of conjugated antibody was added to up to 1 x 10⁶ cells and incubated for 30 minutes at room temperature.
3. 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 Human Lyse Buffer (Catalog # FC002).
4. 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 mouse IgG₂B 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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.