Monoclonal Anti-human Reg1A-APC

Catalog Number: IC4937A
Lot Number: ACKB01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated rat monoclonal anti-human Reg1A: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 431202

Isotype: rat IgG_{2B}

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

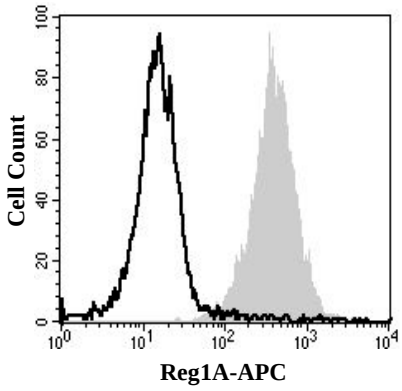
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing Reg1A within a population and qualitatively determine the density of intracellular Reg1A 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 purified, *E. coli*-derived, recombinant human Reg1A (rhReg1A; aa 23 - 166; Accession # P05451). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of Reg1A 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.



AGS cells were stained with APC-conjugated anti-human Reg1A (Catalog # IC4937A, filled histogram) or APC-conjugated isotype control (Catalog # IC0041A, open histogram).

Background Information

Reg1A, also known as PTP, PSP, and lithostathine, is a member of the Reg family of secreted proteins with a C-type lectin domain. Due to variable glycosylation, pancreatic Reg1A exists as multiple species of 16 - 18 kDa. Reg1A promotes the maintenance and growth of pancreatic islet β cells and intestinal villi. It is upregulated in pancreatitis and some carcinomas. Reg1A is an antigenic target in autoimmune diabetes. Human Reg1A shares 65% - 68% amino acid sequence identity with mouse and rat Reg1A.

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. Up to 1 x 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, 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 APC-labeled rat IgG_{2B} 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.

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