Monoclonal Anti-human 5T4-Phycoerythrin

Catalog Number: FAB49751P
Lot Number: ABGL02
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human 5T4: Supplied as 50 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 524744

Isotype: mouse IgG_1

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

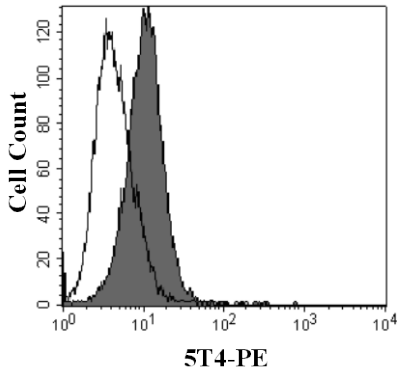
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing 5T4 within a population and qualitatively determine the density of 5T4 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, NS0-derived, recombinant human 5T4 (rh5T4; aa 31 - 355; Accession # Q13641). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of 5T4 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.



BG01V cells were stained with PE-conjugated anti-human 5T4 (Catalog # FAB49751P, filled histogram) or PE-conjugated isotype control (Catalog # IC002P, open histogram).

Background Information

Human 5T4 (oncofetal antigen 5T4; also TPBG and trophoblast glycoprotein) is a 72 kDa glycoprotein member of the LRR family of proteins. It is expressed on trophoblasts, tumor cells, ovarian cuboidal epithelium, and embryonic stem cells, and impacts cell adhesion and motility. The human 5T4 cDNA encodes a type I transmembrane protein precursor that is 420 amino acids (aa) in length. It contains a 324 aa extracellular region (aa 32 - 355) that shows one Ser-rich region followed by seven Leu-rich repeats (aa 90 - 355). Over aa 31 - 355, human 5T4 shares 81% and 85% aa identity with mouse and canine 5T4, respectively.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using BG01V cells.

1. Cells may be Fc-blocked with 1 μg of human IgG/10^5 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 10^6 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 PE-labeled mouse IgG_1 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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