

Reagents Provided

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

Clone #: 549302

Isotype: mouse 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

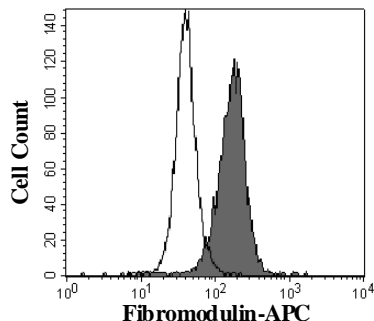
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing Fibromodulin within a population and qualitatively determine the density of intracellular Fibromodulin 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 FMOD (rhFMOD; aa 76 - 376; Accession # NP_002014). 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 Fibromodulin 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.



MG-63 cells were stained with APC-conjugated anti-human Fibromodulin (Catalog # IC5945A, filled histogram) or isotype control (Catalog # IC0041A, open histogram).

Background Information

Fibromodulin (FMOD) is a Class II small leucine-rich proteoglycan (SLRP) superfamily member. The ~50 - 70 kDa protein contains a sulfated N-terminal complement C1q binding domain (aa 19 - 98) and 12 leucine-rich repeats (aa 75 - 376). A 40 kDa form is reported in dental tissues. Fibromodulin participates in collagen fibrillogenesis in many tissues and competes with Lumican for collagen binding. It can participate in inflammation by activating complement. In epidermal keratinocytes, it may be located in the cytoplasm. The region of human FMOD used as an immunogen shares 96% amino acid (aa) identity with mouse or rat FMOD.

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. 5×10^5 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, 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 this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_{2B} 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.

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.