

## Reagents Provided

**Allophycocyanin (APC)-conjugated mouse monoclonal anti-human TM4SF4:** Supplied as 5 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

**Clone #:** 832441

**Isotype:** mouse IgG<sub>1</sub>

## Reagents Not Provided

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

## Storage

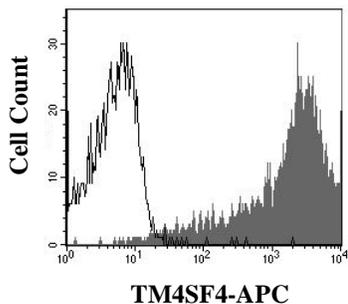
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing TM4SF4 within a population and qualitatively determine the density of TM4SF4 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 NS0 cells transfected with human TM4SF4 (Accession # NP\_004608). 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 TM4SF4 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.



HT-29 cells were stained with APC-conjugated anti-human TM4SF4 (Catalog # FAB7998A; filled histogram) or APC-conjugated isotype control (Catalog # IC002A; open histogram).

## Background Information

TM4SF4 is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. Most of these members are cell-surface proteins that are characterized by the presence of four hydrophobic domains. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth, and motility. TM4SF4 is a cell surface glycoprotein that regulates the adhesive and proliferative status of intestinal epithelial cells. TM4SF4 and matrix metalloproteinase 26 (MMP-26) expression has been found to be significantly decreased during the implantation window in patients with polycystic ovary syndrome.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using HT-29 cells and HEK293/hTM4SF4 transfectants.

- Cells may be Fc-blocked with 1 µg of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL of conjugated antibody was added to up to 1 x 10<sup>6</sup> cells and incubated for 30 minutes at room temperature.
- 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).
- 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 IgG<sub>1</sub> 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.