For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-Human ST2

Code No.	Clone	Subclass	Quantity	Concentration
D066-3	FB9	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: The *ST2* gene, also known as *T1*, *Fit1*, or *DER4*, was originally identified as a responsive gene that was highly induced by stimulation of various proliferation-inducing agents including serum, PDGF (platelet-derived growth factor), FGF (fibroblast growth factor), or lysophosphatidic acid in murine fibroblasts. Three distinct forms of gene products have been reported and named ST2, ST2V, and ST2L.

ST2 is a soluble secreted form of 37 kDa protein, which lacks intracellular domain, whereas ST2L is a transmembrane form of 62 kDa protein (the glycosylated forms of ST2 and ST2L are about 57 and 80 kDa, respectively). This ST2L protein is very similar to IL-1R (interleukin-1 receptor) type I and II in structure, thus it is considered as a member of the IL-1R family. ST2V, which is another novel variant form of human ST2, has been identified recently.

ST2 proteins are expressed in the wide variety types of human cells, including hematopoietic cells in various stages of differentiation, a population of the peripheral blood mononuclear cells from healthy individuals, glioblastoma and astrocytoma cell lines, and colon cancer cells in addition to fibroblast cell lines. Thus ST2 proteins are considered to have some roles in regulating cell growth or proliferation. On the other hand, either definitive functions of ST2 proteins or their ligand molecule(s) which binds to ST2 proteins have remained unclear, though it has been reported that none of IL-1α, β, RA (receptor antagonist) binds to ST2 proteins in spite of their structural similarity to IL-1R. This indicates that ST2L protein is functionally independent from IL-1R. Furthermore, several studies have shown that ST2L is expressed on the cell surface of Th2 cells but not on the Th1 cells, indicating the possibility that ST2L protein participates not only in the regulation of cell growth or proliferation, but also in the immune system including differentiation of T cells or immunological response via helper T cells.

From these observations, ST2 proteins are considered to be one of the important proteins participate in various physiological phenomenon, thus further analysis are required to understand its physiological functions.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone FB9) was established by fusion of mouse myeloma cell PAI with Balb/c mouse splenocyte immunized with the secreted form of ST2 protein which were purified from culture supernatant of COS7 transfectant cells.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human ST2 on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested*
Immunoprecipitation; Not tested*
*It is reported that this monoclonal antibody can be used in Western blotting and Immunoprecipitation in the reference number 3).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)
ELISA; Not tested*
*It is reported that this monoclonal antibody can be used in ELISA in the reference number 2).

Detailed procedure is provided in the following **PROTOCOL**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	transfectant	Not Tested	Not Tested
Reactivity on FCM	+		

INTENDED USE:

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REFERENCES:

1) Tajima, S., *et al., Chest* **124**, 1206-1214 (2003)
2) Haga, Y., *et al., Eur. J. Biochem.* **270**, 163-170 (2003)
3) Kuroiwa, K., *et al., Hybridoma* **19**, 151-159 (2000)
4) Tominaga, S., *et al., BBRC* **264**, 14-18 (1999)
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6) Yanagisawa, K., *et al., J. Biochem.* **121**, 95-103 (1997)
7) Yanagisawa, K., *et al., FEBS Lett.* **318**, 83-87 (1993)
8) Lanahan, A., *et al., Mol. Cell. Biol.***12**, 3919-3929 (1992)
9) Tominaga, S., *et al., FEBS Lett.* **258**, 301-304 (1989)

Clone FB9 is used in reference number 1) - 3).

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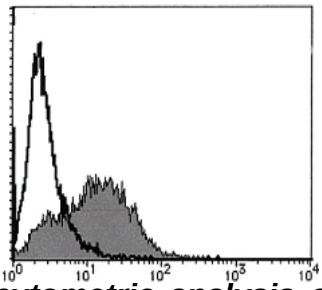
RELATED PRODUCTS:

D065-3 Anti-Human ST2 (HB12)
D067-3 Anti-Human ST2 (2A5)
D067-4 FITC labeled Anti-Human ST2 (2A5)
D067-5 PE labeled Anti-Human ST2 (2A5)



Flow cytometric analysis of human ST2 expression in transfectant. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D066-3 to the cells.*

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 μL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 30 μL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; transfectant)