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

MONOCLONAL ANTIBODY

Anti-Mouse OSMR

Code No.	Clone	Subclass	Quantity	Concentration
D059-3	30-1	Rat IgG2a	100 μg	1 mg/mL

BACKGROUND: Oncostatin M (OSM) is a member of the interleukin-6 (IL-6) family of cytokines that share the gp130 receptor subunit. Mouse OSM (mOSM) plays hematopoietic progenitor cells in the aorta-gonad-mesonephros region of mouse embryo at 11.5 days postcoitum, where definitive hematopoiesis is considered to be initiated in the mouse embryo. Sertoli cells in neonatal testis express mOSM and their proliferation is strongly stimulated by mOSM mOSM-responsive cell lines express high affinity mOSM receptors, as well as mOSMRβ, whereas embryonic stem cells, which are responsive to leukemia inhibitory factor (LIF) but not to mOSM, don't express mOSMRβ. mOSMRβ alone binds mOSM with low affinity and forms a high-affinity receptor with gp130.

SOURCE: This antibody was purified from culture supernatant using protein G agarose. This hybridoma (clone 30-1) was established by fusion of mouse myeloma cell P3X with rat lymphocyte immunized with LO cells*.
*The LO cell line was established from 11.5 dpc mouse embryo as a mOSM-dependent cell line.

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 mouse OSMR on Flow cytometry.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunocytochemistry; Not tested
Immunohistochemistry; Not tested
Flow cytometry; 50-100 μg/mL (final concentration)

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

INTENDED USE:
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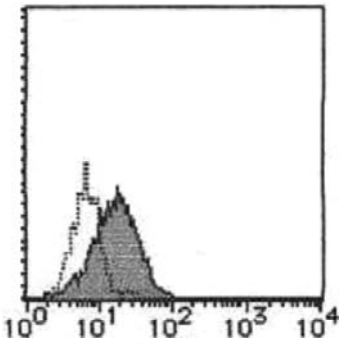
SPECIES CROSS REACTIVITY:

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

REFERENCES:
1) Tanaka, M., *et al., Blood* **93**, 804-815 (1999)
2) Mukouyama, Y., *et al., Immunity* **8**, 105-114 (1998)
3) Hara, T., *et al., Dev. Biol.* **201**, 144-153 (1998)
4) Medvinsky, A., *et al., Cell* **86**, 897-906 (1996)



Flow cytometric analysis of mouse OSMR expression on LO cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D059-3 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 1:40 PE conjugated anti-rat IgG (MBL; code no. IM-1623) 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; LO)