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

MONOCLONAL ANTIBODY

Anti-IL-18 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D046-3	39-3F	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: Interleukin 18 (IL-18) is a 18 kDa cytokine which identified as a costimulatory factor for production of interferon-γ (IFN-γ) in response to toxic shock and shares functional similarities with IL-12. IL-18 is synthesized as a precursor 24 kDa molecule without a signal peptide and must be cleaved to produce an active molecule. IL-1 converting enzyme (ICE, Caspase-1) cleaves pro-IL-18 at aspartic acid in the P1 position, producing the mature, bioactive peptide that is readily released from the cells. It is reported that IL-18 is produced from Kupffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts, adrenal cortex cells and murine diencephalon. IFN-γ is produced by activated T or NK cells and plays critical roles in the defense against microbiral pathogens. IFN-γ activates macrophages, enhances NK activity and B cell maturation, proliferation and Ig secretion, induces MHC class I and II antigens, and inhibits osteoclast activation. IL-18 acts on T helper type-1 (Th1) T cells and in combination with IL-12 strongly induces them to produce IFN-γ. Pleiotropic effects of IL-18 has also been reported, such as, enhancement production of IFN-γ and GM-CSF in peripheral blood mononuclear cells, production of Th1 cytokines, IL-2, GM-CSF and IFN-γ in T cells, enhancement of Fas ligand expression by Th1 cells.

SOURCE: This antibody was purified from mouse ascites fluid using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with SD rat splenocyte immunized with recombinant mouse IL-18.

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 IL-18 on Western blotting.

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

APPLICATIONS:
Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunocytochemistry; Not tested
Immunohistochemistry; Not tested
Flow cytometry; Not tested
Neutralization; Not tested*
*It is reported that this monoclonal antibody can be used in Neutralization in the reference number 5).

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Samples	Recombinant	Recombinant	Not tested
Reactivity on WB	-	+	

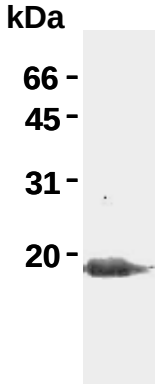
REFERENCES:
1) Venkatachalam, K., *et al.*, *J. Biol. Chem.* **284**, 7853-7865 (2009) [WB]
2) Hoshino, T., *et al.*, *Am. J. Respir. Crit. Care Med.* **176** 49-62 (2007) [WB]
3) Seino, H., *et al.*, *Am. J. Physiol. Gastrointest. Liver Physiol.* **292**, G262-G267 (2007) [WB]
4) Hoshino, T., *et al.*, *J. Immunol.* **166**, 7014-7018 (2001) [WB]
5) Lertmemongkolchai, G., *et al.*, *J. Immunol.* **166**, 1097-1105 (2001) [Neutralization]
6) Dao, T., *et al. Cell Immunol.* **173**, 230-235 (1996)
7) Ushio, S., *et al. J. Immunol.* **156**, 4274-4279 (1996)
8) Micallef, M., *et al. Eur. J. Immunol.* **26**, 1647-1651 (1996)
9) Okamura, H., *et al. Nature* **378**, 88-91 (1995)

This clone is used in the reference number 1) - 5).

PROTOCOL:
SDS-PAGE & Western Blotting
1) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
3) To reduce nonspecific binding, soak the membrane in

10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
4) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
5) Wash the membrane with PBS (5 minutes x 3 times).
6) Incubate the membrane with the 1:10,000 Anti-IgG (Rat) pAb-HRP (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
7) Wash the membrane with PBS (5 minutes x 3 times).
8) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
9) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Recombinant)



Western blot analysis of mouse IL-18 expression in recombinant Mouse IL-18 using D046-3

RELATED PRODUCTS:

Antibodies

D047-3	Anti-IL-18 (Mouse) mAb (74)
D048-3	Anti-IL-18 (Mouse) mAb (93-10C)
D048-6	Anti-IL-18 (Mouse) mAb-Biotin (93-10C)
D043-3	Anti-IL-18 (Human) mAb (25-2G)
D044-3	Anti-IL-18 (Human) mAb (125-2H)
D045-3	Anti-IL-18 (Human) mAb (159-12B)
D045-6	Anti-IL-18 (Human) mAb-Biotin (159-12B)
D306-3	Anti-IL-18 BP (Mouse) mAb (#36)
D307-3	Anti-IL-18 BP (Mouse) mAb (#31)
D304-3	Anti-IL-18 BP (Human) mAb (#36)
D305-3	Anti-IL-18 BP (Human) mAb (#13)
PM014	Anti-IL-18 (Human) pAb
M157-3	Anti-IL-18 (Rat) mAb (21A12)
M158-3	Anti-IL-18 (Rat) mAb (91D8)
M163-3	Anti-IL-18 receptor 1 (Mouse) mAb (33A11)
M166-3	Anti-IL-18 receptor 1 (Mouse) mAb (64G4)
M159-3	Anti-IL-18 receptor 1 (Human) mAb (44G6)
M156-3	Anti-pro-IL-18 (Human) mAb (43A11)

ELISA Kits

7625	Mouse IL-18 ELISA Kit
7620	Human IL-18 ELISA Kit

Recombinant Proteins

B002-5	Recombinant Mouse IL-18
B004-5	Recombinant Mouse IL-18 (without BSA)
B001-5	Recombinant Human IL-18
B003-5	Recombinant Human IL-18 (without BSA)