

MONOCLONAL ANTIBODY

Anti-IL-18 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D047-3	74	Rat IgG2a	100 μ L	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 microbial 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 immunosuppressed golden hamster ascites fluid using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell Y3Ag 1.2.3 with SD rat splenocyte immunized with recombinant mouse IL-18.

FORMULATION: 100 μ g IgG in 100 μ L 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 Immunoprecipitation and ELISA*.
*Suggested paired clone for ELISA is 93-10C (MBL; code no. D048-6)

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

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; 5 μ g/0.5 μ g Mouse IL-18
Immunocytochemistry; Not tested
Immunohistochemistry; Not tested
Flow cytometry; Not tested

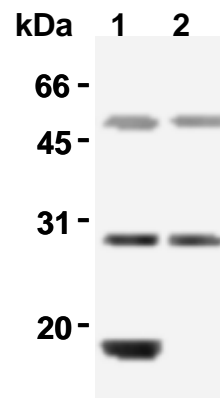
Detailed procedure is provided in the following **PROTOCOL**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Samples	Not tested	Recombinant	Not tested
Reactivity on IP		+	

REFERENCES:

- 1) Abu Elhija, M., *et al.*, *Eur. Cytokine Netw.* **19**, 15-24 (2008) [ELISA]
- 2) Luo, Y., *et al.*, *Clin. Exp. Immunol.* **137**, 24-34 (2004) [WB]
- 3) Dao, T., *et al.*, *Cell Immunol.* **173**, 230-235 (1996)
- 4) Micallef, M., *et al.*, *Eur. J. Immunol.* **26**, 1647-1651 (1996)
- 5) Ushio, S., *et al.*, *J. Immunol.* **156**, 4274-4279 (1996)
- 6) Okamura, H., *et al.*, *Nature* **378**, 88-91 (1995)



Immunoprecipitation of Mouse IL-18 from recombinant protein with D047-3 (1) and rat IgG (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D046-3.

PROTOCOL:

Immunoprecipitation

- 1) Suspend 1 μ g/100 μ L of recombinant Mouse IL-18 with 20 mM phosphate buffer (pH 7.0).

- 2) Add the antibody at the amount of as suggest in the **APPLICATIONS**. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein G agarose beads resuspended in the 20 mM phosphate buffer (pH 7.0). Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 3) Wash the beads 3-5 times with the 20 mM phosphate buffer (pH 7.0)
(centrifuge the tube at 2,500 x g for 10 seconds).
- 4) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-polyacrylamide gel for electrophoresis.
- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 6) 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.
- 7) Incubate the membrane with 1 µg/mL of Anti-IL-18 (Mouse) mAb (MBL; code no. D046-3) diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) 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.
- 10) Wash the membrane with PBS-T (5 minutes x 6 times).
- 11) 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.
- 12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Recombinant mouse IL-18)

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)

RELATED PRODUCTS:

Antibodies

D047-3	Anti-IL-18 (Mouse) mAb (74)
D046-3	Anti-IL-18 (Mouse) mAb (39-3F)
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)