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

MBL
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MONOCLONAL ANTIBODY

Anti-IL-18 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D047-3	74	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 hybridoma (clone 74) supernatant 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 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 Immunoprecipitation and ELISA*.
*Suggested paired clone for ELISA is 93-10C (MBL; code no. D048-6)

INTENDED USE:
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APPLICATIONS:
Western blotting; Not recommended
Immunoprecipitation; 5 µg/0.5 µg Mouse IL-18
Immunocytochemistry; Not tested
Immunohistochemistry; Not tested
Flow cytometry; Not tested

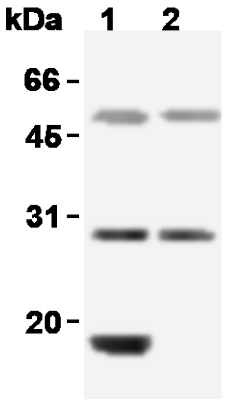
Detailed procedure is provided in the following **PROTOCOL**.

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) Dao, T., *et al., Cell Immunol.* **173**, 230-235 (1996)
3) Micallef, M., *et al.*, *Eur. J. Immunol.* **26**, 1647-1651 (1996)
4) Ushio, S., *et al., J. Immunol.* **156**, 4274-4279 (1996)
5) Okamura, H., *et al.*, *Nature* **378**, 88-91 (1995)

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



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.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:

Immunoprecipitation

1) Suspend 1 µg/100 µL 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/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.
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 HRP-conjugated anti-rat IgG antibody 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)

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