

MONOCLONAL ANTIBODY

Anti-IL-18 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D048-3	93-10C	Rat IgG1	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 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 hybridoma (clone 93-10C) supernatant 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. 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.

SPECIES CROSS REACTIVITY:

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

INTENDED USE:

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

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; 5 µg/0.5 µg recombinant mouse IL-18.
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Neutralization; Induction of IFN- γ by mouse IL-18 receptor transfected KG-1 cell (KG-1 cell: Human myelomonocyte: ATCC CCL246) in response to the 30 ng/mL recombinant mouse IL-18 was neutralized by this antibody. The neutralization activity of 010 is as follows;

Antibody concentration	Inhibition dose*
0.5 µg/mL	> 50%
5 µg/mL	> 90%

*Neutralization activity can be varied depends on cell conditions, IL-18 concentration.

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

REFERENCES:

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- 3) Humann, J. and Lenz, L. L., *J. Immunol.* **184**, 5172-5178 (2010) [NT]
- 4) Chaix, J., *et al.*, *J. Immunol.* **181**, 1627-1631 (2008) [NT]
- 5) Beadling, C. and Slifka, M. K., *Blood* **105**, 1179-1186 (2005) [NT]
- 6) Wang, S. Z., *et al.*, *J. Immunol.* **173**, 4040-4049 (2004) [NT]
- 7) Malmgaard, L. and Paludan, S. R., *J. Gen. Virol.* **84**, 2497-2500 (2003) [NT]
- 8) Fukao, T., *et al.*, *J. Immunol.* **164**, 64-71 (2000) [NT]
- 9) Dao, T., *et al.*, *Cell Immunol.* **173**, 230-235 (1996)
- 10) Micallef, M., *et al.*, *Eur. J. Immunol.* **26**, 1647-1651 (1996)
- 11) Ushio, S., *et al.*, *J. Immunol.* **156**, 4274-4279 (1996)
- 12) Okamura, H., *et al.*, *Nature* **378**, 88-91 (1995)

As this antibody is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.

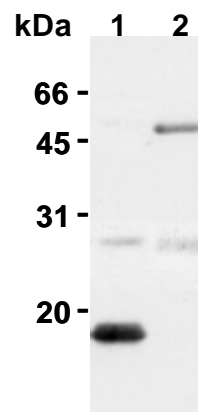
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

Immunoprecipitation

- 1) Suspend 1 µg/100 µL of recombinant Mouse IL-18 with 20 mM phosphate buffer (pH 7.0).
- 2) Add Anti-IL-18 (Mouse) mAb (D048-3) or Rat IgG1 (isotype control) (M080-3) 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.
- 5) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
- 6) 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% methanol). See the manufacture's manual for precise transfer procedure.
- 7) 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.
- 8) Incubate the membrane with 0.5 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.)
- 9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6).
- 10) 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.
- 11) Wash the membrane with PBS-T (5 minutes x 6).
- 12) 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.
- 13) 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)



Immunoprecipitation of Mouse IL-18 from recombinant protein with D048-3 (1) and rat IgG (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D046-3.

Neutralization

Neutralization activity of the antibody can be varied depends on cell types and growth conditions.

Neutralization activity for this antibody is defined as that concentration of the antibody required to inhibit recombinant Mouse IL-18 bioactivity on mouse IL-18 receptor transfected KG-1 cells with the following conditions;

- 1) Mouse IL-18 receptor transfected KG-1 cells were cultured at 3x10⁵ cells/mL for 4 days at 37°C in 5% CO₂ incubator with RPMI 1640.
- 2) After 2 days of pre-culture, the cell concentration was adjusted to 2x10⁶ cells/mL and incubated for 46-48 hours at 37°C in 5% CO₂ incubator with RPMI 1640 in the presence of anti-Mouse IL-18 antibody diluted as suggested in APPLICATIONS and 30 ng/mL of Mouse IL-18.
- 3) The culture supernatant was recovered and the amount of IFN-γ were measured by IFN-γ ELISA Kit (MBL; code no. IM-1743).

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