

Anti-IL-18 (Mouse) mAb (Functional Grade)

CODE No.	D048-3M2
CLONALITY	Monoclonal
CLONE	93-10C
ISOTYPE	Rat IgG1
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Mouse IL-18 (recombinant)
FORMULATION	PBS. Azide free, 0.22 µm sterile-filtered Endotoxin level is < 0.5 EU/mg antibody, as determined by the LAL assay.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

<u>Immunoprecipitation</u>	2 µg/0.2 µg recombinant mouse IL-18
<u>Functional activity</u>	0.5-5 µg/mL for 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 lot 001 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.

SPECIES CROSS REACTIVITY on IP

Species	Human	Mouse	Rat	Hamster
Sample	Not tested	Recombinant protein	Not tested	Not tested
Reactivity		+		

Entrez Gene ID 16173 (Mouse)

- REFERENCES**
- 1) Abdel Fattah, E., *et al.*, *J. Immunol.* **194**, 5407-5416 (2015)
 - 2) Hirano, Y., *et al.*, *Nat. Med.* **20**, 1372-1375 (2014)
 - 3) Zanoni, I., *et al.*, *Cell Rep.* **4**, 1235-1249 (2013)
 - 4) Rogers, T. B., *et al.*, *J. Mol. Cell Cardiol.* **50**, 346-356 (2011)
 - 5) Dao, T., *et al.*, *Cell Immunol.* **173**, 230-235 (1996)
 - 6) Micallef, M., *et al.*, *Eur. J. Immunol.* **26**, 1647-1651 (1996)
 - 7) Ushio, S., *et al.*, *J. Immunol.* **156**, 4274-4279 (1996)
 - 8) Okamura, H., *et al.*, *Nature* **378**, 88-91 (1995)

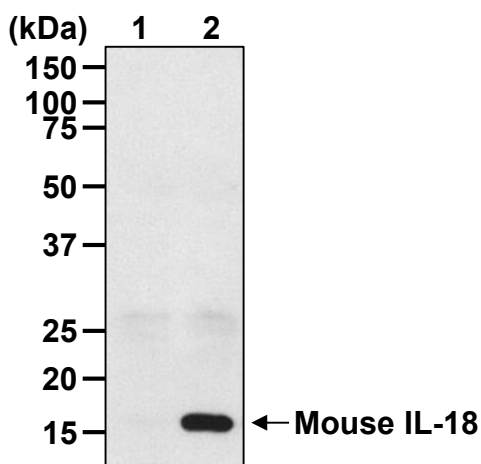
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Immunoprecipitation

- 1) Suspend 200 ng/100 μ L of recombinant Mouse IL-18 with PBS containing 1% BSA and 0.09% NaN₃.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 2) Add the antibody at the amount of as suggest in the **APPLICATIONS**. Mix well and incubate with gentle agitation for 1 hr. at 4°C.
- 3) Add 20 μ L of 50% protein G agarose beads resuspended in PBS. Mix well and incubate with gentle agitation for 1 hr. at 4°C.
- 4) Wash the beads 5 times with 1 mL of PBS.
- 5) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 5 min., and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
- 7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 8) Incubate the membrane with 1 μ g/mL of the Anti-IL-18 (Mouse) mAb (MBL; code no. D046-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody to be used will depend on conditions.)
- 9) Wash the membrane with PBS (5 min. x 3 times).
- 10) Incubate the membrane with 1:5,000 of HRP-conjugated anti-rat IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 11) Wash the membrane with PBS (5 min. x 3 times).
- 12) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. 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 1 min. 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

- Lane 1: Rat IgG1 (isotype control) (M080-3)
Lane 2: Anti-IL-18 (Mouse) mAb (D048-3M2)

Immunoblotted with Anti-IL-18 (Mouse) mAb (MBL; code no. 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) Culture the mouse IL-18 receptor transfected KG-1 cells at 3×10^6 cells/mL in serum-free RPMI 1640 medium for 1 day at 37°C in 5% CO₂ incubator.
- 2) Adjust the cell concentration to 2×10^6 cells/mL by serum-free RPMI1640 medium.
- 3) Incubate the cells for 1 day at 37°C in 5% CO₂ incubator with RPMI 1640 medium in the presence of Anti-IL-18 (Mouse) mAb (D048-3M2) diluted as suggested in the **APPLICATIONS** and 30 ng/mL of Recombinant Mouse IL-18 (without BSA) (MBL; code no. B004-5).
- 4) Harvest the culture supernatant and measure the amount of IFN- γ by Quantikine IFN- γ ELISA Kit (R&D Systems; code no. DIF50).

(Positive control for Neutralization; Transfectant)

Concentration of Anti-IL18 (Mouse) mAb	Inhibition rate	Criteria
0.5 μ g/mL	81.6%	> 50%
5 μ g/mL	97.6%	> 90%