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MBL
A JSR Life Sciences Company

Anti-IL-18 (Human) mAb

(Functional Grade)

CODE No. D044-3M2

CLONALITY Monoclonal
CLONE 125-2H
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Human IL-18 (recombinant)
FORMULATION 0.1 M NaPB (pH 6.0)/0.15 M NaCl. 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

Immunoprecipitation 2 µg/0.2 µg recombinant human IL-18

Functional activity 0.1-1 µg/mL for neutralization

Induction of IFN-γ by human IL-18 receptor expressed on KG-1 cell (KG-1 cell: Human myelomonocyte: ATCC CCL246) in response to the 40 ng/mL recombinant human IL-18 was neutralized by this antibody. The neutralization activity of lot 001 is as follows;

Antibody concentration	Inhibition dose*
0.1 µg/mL	> 50%
1 µg/mL	> 90%

*Neutralization activity can be varied depends on cell conditions.

SPECIES CROSS REACTIVITY on IP

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

Entrez Gene ID 3606 (Human)

REFERENCES

1) Nussbaumer, O., *et al., Blood* **118,** 2743-2751 (2011)
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3) Wu, C., *et al., J. Immunol.* **170**, 5571-5577 (2003)
4) Sugawara, S., *et al., J. Immunol.* **167**, 6568-6575 (2001)
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)
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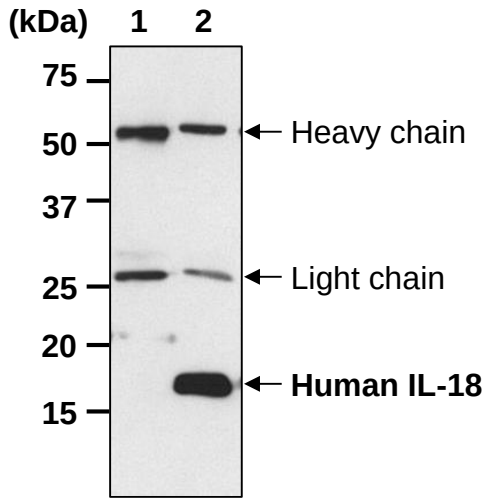
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Immunoprecipitation

1) Mix 20 µL of 50% protein G agarose beads slurry resuspended in PBS with Mouse IgG1 (isotype control) (MBL, code no. M075-3M2) or Anti-IL-18 (Human) mAb (D044-3M2) at the amount as suggested in the **APPLICATIONS**, and then add 1 mL of Wash buffer I [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] into each tube. Incubate with gentle agitation for 1 hr. at room temperature.
2) Wash the beads twice with 1 mL of Wash buffer I.
3) Add 200 ng of recombinant human IL-18 in 100 µL of PBS containing 1% BSA and 0.09% NaN_3.
   *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.
4) Incubate with gentle agitation for 1 hr. at room temperature.
5) Wash the bead pellet 5 times with 1 mL of Wash buffer II [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.05% NP-40].
6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 5 min., and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
8) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Incubate the membrane with 1 µg/mL of the Anti-IL-18 (Human) mAb (MBL, code no. D043-3) 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.)
10) Wash the membrane with PBS (5 min. x 3 times).
11) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 300) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
12) Wash the membrane with PBS (5 min. x 3 times).
13) 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.
14) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.
    (Positive control for Immunoprecipitation; Recombinant human IL-18)



Immunoprecipitation of human IL-18 from recombinant protein
Lane 1: Mouse IgG1 (isotype control) (MBL, code no. M075-3)
Lane 2: Anti-IL-18 (Human) mAb (MBL, code no. D044-3M2)

Immunoblotted with Anti-IL-18 (Human) mAb (MBL, code no. D043-3)

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 human IL-18 bioactivity on KG-1 cells with the following conditions;

1) Prepare KG-1 cells at 3 x 10^6 cells/mL in RPMI 1640 medium with 10% fetal calf serum.
2) Incubate the cells for 1 day at 37°C in 5% CO_2 incubator in the presence of Anti-IL-18 (Human) mAb (D044-3M2) diluted as suggested in the **APPLICATIONS** and 40 ng/mL of Recombinant Human IL-18 (without BSA) (MBL, code no. B003-5).
3) 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; KG-1)

Concentration of Anti-IL18 (Human) mAb	Inhibition rate	Criteria
0.1 μg/mL	84.2%	> 50%
1 μg/mL	92.6%	> 90%