

MONOCLONAL ANTIBODY

Anti-IL-18 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D044-3	125-2H	Mouse IgG1 κ	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 hybridoma (clone 125-2H) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with recombinant human 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 human IL-18 on Immunoprecipitation.

ENDOTOXIN LEVEL: Less than 10 ng/1 mL of antibody, measured by LAL method.

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 recombinant human IL-18

Immunocytochemistry; Not tested*

*It is reported that this antibody can be used in this application in the reference number 4).

Immunohistochemistry; Not tested

Flow cytometry; Not tested

ELISA; Suggested paired clone for ELISA is 159-12B.

Neutralization; Induction of IFN- γ by 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 is as follows;

Antibody concentration	Inhibition dose*
0.1 μ g/mL	> 50%
1.0 μ g/mL	> 90%

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

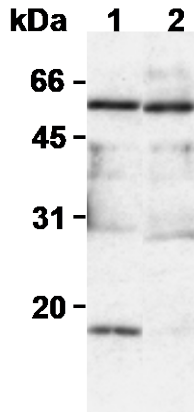
Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

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

REFERENCES:

- 1) Okano, M., *et al.*, *Int. Arch. Allergy Immunol.* **160**, 275-286 (2013) [ELISA]
- 2) Nussbaumer, O., *et al.*, *Blood* **118**, 2743-2751 (2011) [NT]
- 3) Tu, Z., *et al.*, *J. Exp. Med.* **205**, 233-244 (2008) [NT]
- 4) Skurk, T., *et al.*, *Eur. J. Endocrinol.* **152**, 863-868 (2005) [IC]
- 5) Sugawara, S., *et al.*, *J. Immunol.* **167**, 6568-6575 (2001) [NT]
- 6) Shida, K., *et al.*, *J. Immunol.* **166**, 6671-6669 (2001) [ELISA]
- 7) Dao, T., *et al.*, *Cell Immunol.* **173**, 230-235 (1996)
- 8) Micallef, M., *et al.*, *Eur. J. Immunol.* **26**, 1647-1651 (1996)
- 9) Ushio, S., *et al.*, *J. Immunol.* **156**, 4274-4279 (1996)
- 10) Okamura, H., *et al.*, *Nature* **378**, 88-91 (1995)



Immunoprecipitation of Human IL-18 from recombinant protein with D044-3 (1) and Mouse IgG (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D043-3.

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

PROTOCOLS:

Immunoprecipitation

- 1) Suspend 0.5 µg/100 µL of recombinant Human IL-18 with 20 mM phosphate buffer (pH 7.0).
- 2) Add the antibody at the amount as suggested in the **APPLICATIONS**. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A 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) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 4) Resuspend the agarose with 20 mM phosphate buffer (pH 7.0).
- 5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 6) Repeat steps 4)-5) 2-4 times.
- 7) 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.
- 8) 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.
- 9) 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.
- 10) Incubate the membrane with 1 µg/mL of Anti-IL-18 (Human) mAb (MBL, code no. D043-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room

temperature. (The concentration of antibody will depend on the conditions.)

- 11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 12) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 13) Wash the membrane with PBS-T (5 minutes x 6).
- 14) 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.
- 15) 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 human IL-18)

Neutralization

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

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

- 1) KG-1 cells were cultured at 3 x 10⁵ cells/mL for 4 days at 37°C in 5% CO₂ incubator with RPMI 1640 containing 10% fetal calf serum.
- 2) After 4 days of preculture, the cell concentration was adjusted to 3 x 10⁶ cells/mL and incubated for 24 hours at 37°C in 5% CO₂ incubator with RPMI 1640 containing 10% fetal calf serum in the presence of Anti-IL-18 (Human) mAb (MBL, code no. D044-3) diluted as suggested in the **APPLICATIONS** and 40 ng/mL of Human IL-18.
- 3) The culture supernatant was recovered and the amount of IFN-γ were measured by Quantikine IFN-γ ELISA Kit (R&D Systems, code no. DIF50).

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