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

MONOCLONAL ANTIBODY

Anti-Rat IL-18

Code No.	Clone	Subclass	Quantity	Concentration
M158-3	91D8	Mouse IgG2b κ	100 μg	1 mg/mL

BACKGROUND: Interleukin 18 (IL-18) is an 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 and enhances NK activity and B cell maturation, proliferation and Ig secretion. IFN-γ also induces expression of 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 have 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 91D8) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the mature rat IL-18 fusion protein corresponding to 37-194 aa.

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 rat IL-18 on Immunoprecipitation.

INTENDED USE:
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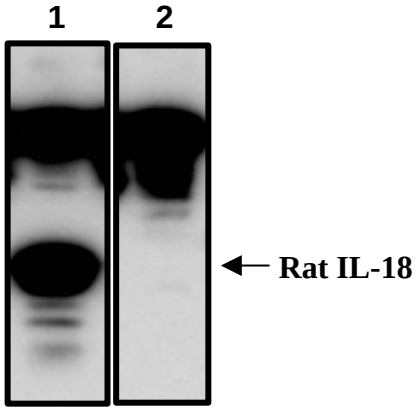
APPLICATIONS:
Western blotting; Not recommended*
*M157-3 is suitable for this application.
Immunoprecipitation; 1 μg/sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Rat	Mouse	Human
Other	recombinant	Not Tested	Not Tested
Reactivity on IP	+		

REFERENCES:
1) Halpern, M. D., *et al.*, *Pediatr Res*. **51**, 733-739 (2002)
2) Jordan, J. A., *et al.*, *J. Immunol*. **167**, 7060-7068 (2001)
3) Culhane, A. C., *et al.*, *Mol. Psychiatry* **3**, 362-366 (1998)



Immunoprecipitation of recombinant mature rat IL-18 with M158-3 (1) or normal mouse IgG (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-rat IL-18 monoclonal antibody (MBL; code no. M157-3).*

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PROTOCOL:

Immunoprecipitation

1) Add primary antibody as suggest in the **APPLICATIONS** into the recombinant protein. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
2) Add 10 µL of 50% protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
3) Wash the beads 3-5 times with the cold Lysis buffer (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/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.
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 1 µg/mL of anti-rat IL-18 monoclonal antibody (Clone 21A12, MBL; code no. M157-3) as primary antibody 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 3 times).
10) Incubate the membrane with the 1:1,000 mouse True Blot (e-bioscience; code no. 8877) 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 times).
12) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
13) 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 2 minutes.
15) Develop the film as usual. The condition for exposure and development may vary.

RELATED PRODUCTS:

M157-3	anti-rat IL-18 (21A12)
M158-3	anti-rat IL-18 (91D8)
PM014	anti-human IL-18 (polyclonal)
D044-3	anti-human IL-18 (125-2H)
D045-3	anti-human IL-18 (159-12B)
D045-6	Biotin labeled anti-human IL-18 (159-12B)
D048-3	anti-mouse IL-18 (93-10C)
D048-6	Biotin labeled anti-mouse IL-18 (93-10C)
D043-3	anti-human IL-18 (25-2G)
D046-3	anti-mouse IL-18 (39-3F)
D047-3	anti-mouse IL-18 (74)
D112-3	anti-IFN-γ (#6)
B001-5	Recombinant human IL-18
B002-5	Recombinant mouse IL-18
B003-5	Recombinant human IL-18 (without BSA)
B004-5	Recombinant mouse IL-18 (without BSA)
7620	Human IL-18 ELISA Kit
7625	Mouse IL-18 ELISA Kit