**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 mouse ascites fluid 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 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 human IL-18 on Immunoprecipitation.

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

**APPLICATIONS:**
- Western blotting: Not recommended
- Immunoprecipitation: 5 µg/0.5 µg recombinant human IL-18
- Immunocytochemistry: Not tested
- Immunohistochemistry: Not tested
- Flow cytometry: Not tested

**REFERENCES:**

This clone is used in reference number 1-4).

**PROTOCOLS:**

**Immunoprecipitation**
1) Suspend 0.5 µg/100µL of recombinant Human IL-18 with 20 mM phosphate buffer (pH 7.0).
2) Add the antibody 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 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) 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.
Use 10 μL/lane for the SDS-polyacrylamide gel for
electrophoresis.
5) 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% MeOH). See the manufacture’s manual for precise
transfer procedure.
6) 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.
7) Incubate the membrane with 1 μg/mL of the anti-Human
IL-18 antibody (MBL; code no. D043-3) diluted with
PBS, pH 7.2 containing 1% skimmed milk for 1 hour at
room temperature. (The concentration of antibody will
depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in
PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000
HRP-conjugated anti-Mouse IgG (MBL; code no. 330)
diluted with 1% skimmed milk (in PBS, pH 7.2) for 1
hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) 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.
12) 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 that
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 3×10⁶ 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 3×10⁶ 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-Human IL-18
antibody diluted as suggested in APPLICATIONS and
40 ng/mL of Human IL-18.
3) The culture supernatant were recovered and the amount
of IFN-γ were measured by IFN-γ ELISA Kit (MBL;
code no. IM-1743).

RELATED PRODUCTS:
Antibodies
D043-3  Anti-IL-18 (Human) mAb (25-2G)
D045-3  Anti-IL-18 (Human) mAb (159-12B)
D045-6  Anti-IL-18 (Human) mAb-Biotin (159-12B)
D304-3  Anti-IL-18 BP (Human) mAb (#36)
D305-3  Anti-IL-18 BP (Human) mAb (#13)
D306-3  Anti-IL-18 BP (Mouse) mAb (#36)
D307-3  Anti-IL-18 BP (Mouse) mAb (#31)
PM014  Anti-IL-18 (Human) pAb
D046-3  Anti-IL-18 (Mouse) mAb (39-3F)
D047-3  Anti-IL-18 (Mouse) mAb (74)
D048-3  Anti-IL-18 (Mouse) mAb (93-10C)
D048-6  Anti-IL-18 (Mouse) mAb-Biotin (93-10C)
M157-3  Anti-IL-18 (Rat) mAb (21A12)
M158-3  Anti-IL-18 (Rat) mAb (91D8)
M159-3  Anti-IL-18 receptor 1 (Human) mAb (44G6)
M163-3  Anti-IL-18 receptor 1 (Mouse) mAb (33A11)
M166-3  Anti-IL-18 receptor 1 (Mouse) mAb (64G4)
M156-3  Anti-pro-IL-18 (Human) mAb (43A11)

ELISA Kits
7620  Human IL-18 ELISA Kit
7625  Mouse IL-18 ELISA Kit

Recombinant Proteins
B001-5  Recombinant Human IL-18
B003-5  Recombinant Human IL-18 (without BSA)
B002-5  Recombinant Mouse IL-18
B004-5  Recombinant Mouse IL-18 (without BSA)