

MONOCLONAL ANTIBODY

# Anti-IL-18 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D043-3	25-2G	Mouse IgG1 $\kappa$	100 $\mu$ L	1 mg/mL

**BACKGROUND:** Interleukin 18 (IL-18) is an 18 kDa cytokine which identified as a costimulatory factor for production of interferon- $\gamma$  (IFN- $\gamma$ ) 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- $\gamma$  is produced by activated T or NK cells and plays critical roles in the defense against microbial pathogens. IFN- $\gamma$  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- $\gamma$ . Pleiotropic effects of IL-18 has also been reported, such as, enhancement production of IFN- $\gamma$  and GM-CSF in peripheral blood mononuclear cells, production of Th1 cytokines, IL-2, GM-CSF and IFN- $\gamma$  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  $\mu$ g IgG in 100  $\mu$ 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 Western blotting.

**SPECIES CROSS REACTIVITY:**

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

**APPLICATIONS:**

Western blotting; 1  $\mu$ g/mL for chemiluminescence detection system

Immunoprecipitation; Not tested

Immunocytochemistry; Not tested

Immunohistochemistry; Not tested\*

\*It is reported that this monoclonal antibody can be used in Immunohistochemistry for paraffin section in the reference number 3)-5), and for frozen section in the reference number 4).

Flow cytometry; Not tested

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

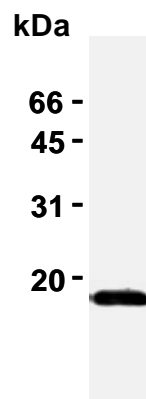
**INTENDED USE:**

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

**REFERENCES:**

- 1) Guo, H., *et al.*, *Mediators Inflamm.* **2018**, 7304096 (2018) [WB]
- 2) Li, W. L., *et al.*, *Arc. Med. Sci.* **12**, 950-958 (2016) [WB]
- 3) Walsh, J. G., *et al.*, *Retrovirology* **11**, 35 (2014) [IHC]
- 4) Sakai, A., *et al.*, *J. Immunol.* **181**, 2898-2906 (2008) [IHC]
- 5) van Oosterhout, M., *et al.*, *Ann. Rheum. Dis.* **64**, 537-543 (2005) [IHC]
- 6) Takiyama, Y., *et al.*, *Thyroid* **12**, 935-943 (2002) [IHC]
- 7) Shida, K., *et al.*, *J. Immunol.* **166**, 6671-6679 (2001)
- 8) Akita, K., *et al.*, *J. Biol. Chem.* **272**, 26595-26603 (1997)
- 9) Dao, T., *et al.*, *Cell Immunol.* **173**, 230-235 (1996)
- 10) Micallef, M., *et al.*, *Eur. J. Immunol.* **26**, 1647-1651 (1996)
- 11) Ushio, S., *et al.*, *J. Immunol.* **156**, 4274-4279 (1996)
- 12) Okamura, H., *et al.*, *Nature* **378**, 88-91 (1995)

Clone 25-2G is used in reference number 1) - 8).



**Western blot analysis of Human IL-18 expression in recombinant Human IL-18 using D043-3**

## PROTOCOL:

### **SDS-PAGE & Western Blotting**

- 1) Boil the samples for 2 minutes and centrifuge. Load 10  $\mu$ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 3) 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.
- 4) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 6) 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.
- 7) Wash the membrane with PBS-T (10 minutes x 3 times).
- 8) 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.
- 9) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Recombinant)

## RELATED PRODUCTS:

### Antibodies

D044-3	Anti-IL-18 (Human) mAb (125-2H)
D045-3	Anti-IL-18 (Human) mAb (159-12B)
D045-6	Anti-IL-18 (Human) mAb-Biotin (159-12B)
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)

### 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)