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

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MONOCLONAL ANTIBODY

Anti-IL-18 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D043-3	25-2G	Mouse IgG1 κ	100 µL	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, 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 Western blotting.

SPECIES CROSS REACTIVITY:

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

APPLICATIONS:

Western blotting; 1 µg/mL
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:

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REFERENCES:

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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

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

PROTOCOL:

SDS-PAGE & Western Blotting

1) Boil the samples for 2 minutes and centrifuge. Load 10 μL 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^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
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).
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)

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