**MONOCLONAL ANTIBODY**  

**Anti-IL-18 (Human) mAb**

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Clone</th>
<th>Subclass</th>
<th>Quantity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D043-3</td>
<td>25-2G</td>
<td>Mouse IgG1 κ</td>
<td>100 μL</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>

**BACKGROUND:** Interleukin 18 (IL-18) is an 18 kDa cytokine which is 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 Western blotting.

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

**APPLICATIONS:**  
- Western blotting: 1 μg/mL for chemiluminescence detection system  
- Immunoprecipitation: Not tested

**SPECIES CROSS REACTIVITY:**

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>others</td>
<td>recombinant</td>
<td>recombinant</td>
<td>Not Tested</td>
</tr>
</tbody>
</table>

**REFERENCE:**
- Not tested
- Immunohistochemistry: Not tested*
- *It is reported that this monoclonal antibody can be used in Immunohistochemistry for paraffin section in the reference number 2), and for frozen section in the reference number 1).
- Flow cytometry: Not tested

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

**REFERENCES:**

Clone 25-2G is used in reference number 1) - 4).
**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/cm² 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 PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on the condition.)

5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).

6) 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.

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)
- 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)