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>D044-3</td>
<td>125-2H</td>
<td>Mouse IgG1 κ</td>
<td>100 μL</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>

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 hybridoma (clone 125-2H) supernatant 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*
Flow cytometry: Not tested
ELISA: Suggested paired clone for ELISA is 159-12B.
Neutralization: Induction of IFN-γ by KG-1 cell (Human myelomonocyte: ATCC CCL246) in response to the 40 ng/mL recombinant Human IL-18 was neutralized by this antibody. The neutralization activity is as follows;

Antibody concentration  Inhibition dose*  
0.1 μg/mL               > 50%         
1.0 μg/mL               > 90%         

*Neutralization activity can be varied depends on cell conditions, IL-18 concentration.

Detailed procedure is provided in the following PROTOCOLS.

SPECIES CROSS REACTIVITY:

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Recombinant</td>
<td>Recombinant</td>
<td>Not tested</td>
</tr>
<tr>
<td>Reactivity on IP</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES:
2) Nussbaumer, O., et al., Blood 118, 2743-2751 (2011) [NT]
Immunoprecipitation of Human IL-18 from recombinant protein with D044-3 (1) and Mouse IgG (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D043-3.

**APPLICATIONS**

Add the antibody (Anti-human IL-18, MBL, code no. D044-3) diluted in the supernatant. Incubate the membrane with 1:1 PBS [0.05% Tween-20 in PBS] (5 minutes x 3).

**Neutralization**

Neutralization activity of the antibody can be varied depends on cell types and growth conditions.

Neutralization activity for this antibody is defined as the 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 x 10^6 cells/mL for 4 days at 37°C in 5% CO_2 incubator with RPMI 1640 containing 10% fetal calf serum.

2) After 4 days of preculture, the cell concentration was adjusted to 3 x 10^6 cells/mL and incubated for 24 hours at 37°C in 5% CO_2 incubator with RPMI 1640 containing 10% fetal calf serum in the presence of Anti-IL-18 (Human) mAb (MBL, code no. D044-3) diluted as suggested in the APPLICATIONS and 40 ng/mL of Human IL-18.

3) The culture supernatant were recovered and the amount of IFN-γ were measured by Quantikine IFN-γ ELISA Kit (R&D Systems, code no. DIF50).

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