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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-IL-33 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M138-3	5H1	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Interleukin-1 (IL-1) family, such as IL-1α/β and IL-18, have important functions in host defense, immune regulation, and inflammation. IL-33, a member of the IL-1 family, that shows to induce T helper (Th) type 2 responses by signaling through the IL-1 receptor-related protein ST2 (IL-1R4), an orphan member of the IL-1 receptor family. Similarly to IL-1α/β and IL-18, IL-33 is synthesized as a 31 kDa precursor protein has been shown to be cleaved by caspase-1 *in vitro*. *In vivo*, IL-33 induces the expression of IL-4, IL-5, and IL-13 and leads to severe pathological changes in mucosal organs. IL-33 has been originally identified as NF-HEV, which is a nuclear factor preferentially expressed in high endothelial venules. IL-33 may function as both a proinflammatory cytokine and an intracellular nuclear factor involved in transcriptional regulation.

SOURCE: This antibody was purified from hybridoma (clone 5H1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with the recombinant full-length human IL-33 (270 aa).

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-33 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1-2 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/100 ng of recombinant protein
Immunohistochemistry; Not Tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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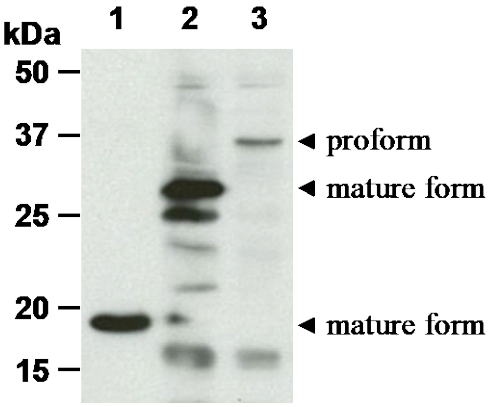
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	recombinant protein transfectant	transfectant	Not Tested
Reactivity on WB	+	-	

REFERENCES:

1) Hayakawa, H., *et al., J. Biol. Chem.* **282**, 26369-26380 (2007)
2) Carriere, V., *et al., PNAS* **104**, 282-287 (2007)
3) Sanada, S., *et al., J. Clin. Invest.* **117**, 1538-1549 (2007)
4) Schmitz, J., *et al.*, *Immunity* **23**, 479-490 (2005)
5) Dinarello, C. A., *Immunity* **23**, 461-462 (2005)
6) Baekkevold, E. S., *et al., Am. J. Pathol.* **163**, 69-79 (2003)



Western blot analysis of IL-33 using M138-3. Lane 1: recombinant human IL-33 (112-270 a.a.) purified protein. Lane 2: Igκ leader sequence-myc-His-human IL-33 (112-270 a.a.) expressed in 293T. Lane 3: myc-human IL-33 (full length) expressed in 293T.

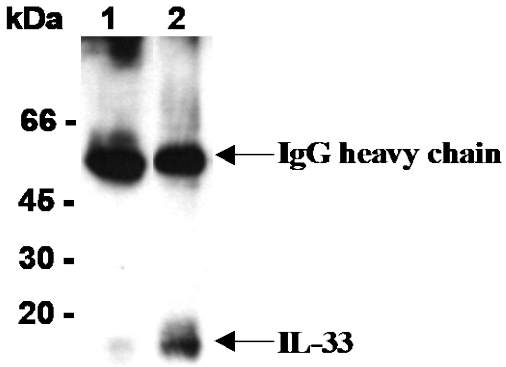
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the 1 x 10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) 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 manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5%

skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) 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.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) 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.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) 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.
10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; transfectant, Jurkat, HeLa, HL-60, A431, 293T, U937, HUVEC)



Immunoprecipitation of human IL-33 from recombinant protein with mouse IgG1 (1) or M138-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M138-3.*

Immunoprecipitation

1) Add primary antibody as suggested in the **APPLICATIONS** into 100 ng of protein. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
2) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
3) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
4) Resuspend the beads with cold Lysis buffer.
5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Repeat steps 4)-5) 2-4 times.
7) 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-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitaion; recombinant)

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