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

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-IL-33 (Human) pAb

Code No.	Quantity	Form
PM033	100 µL	Affinity Purified

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 rabbit serum using affinity column. The rabbit was immunized with the recombinant full-length human IL-33 (270 aa).

FORMULATION: 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 Immunohistochemistry.

APPLICATIONS:
Western blotting; 1:500
Immunoprecipitation; Not recommended
Immunohistochemistry; 1:500 - 1:1,000
Heat treatment is necessary for paraffin embedded sections.
Autoclave; 10 minutes at 110°C in 10 mM citrate buffer (pH 6.5)
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 2).
Flow cytometry; Not tested

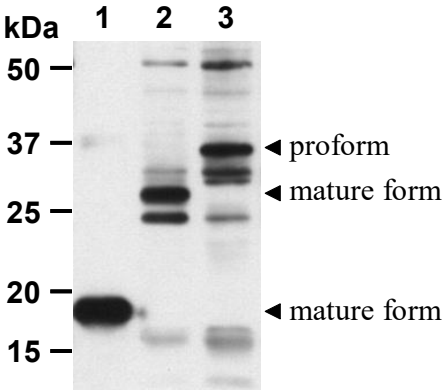
Detailed procedure is provided in the following **PROTOCOLS**.

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Recombinant protein transfectant	Not tested	Not tested
Reactivity on WB	+		

REFERENCES:
1) Guo, Z., *et al., J. Asthma* **51**, 863-869 (2014) [IHC]
2) Nomura, K., *et al., Laryngoscope* **122**, 1185-1192 (2012) [WB, IC]
3) Matsuda, A., *et al*., *Invest. Ophthalmol. Vis. Sci.* **50**, 4646-4652 (2009) [IHC]
4) Schmitz, J., *et al*., *Immunity* **23**, 479-490 (2005)



Western blot analysis of IL-33 using PM033. *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.*

The descriptions of the following protocols are examples.

Each user should determine the appropriate condition.

PROTOCOLS:

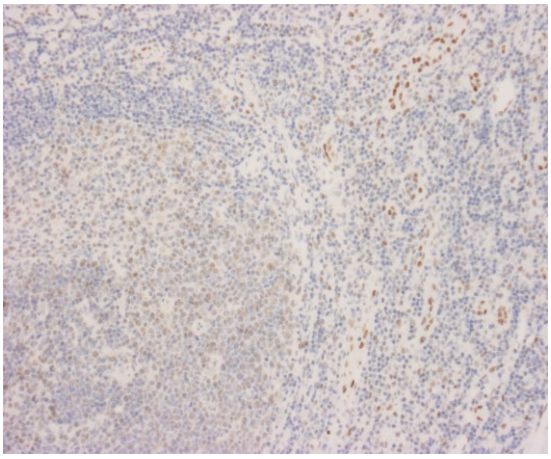
SDS-PAGE & Western blotting

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 20 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).
7) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL, code no. 458) 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).
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 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; transfectant, 293T, Raji, HeLa, HUVEC, Lu99A)



Immunohistochemical detection of IL-33 on human tonsil paraffin embedded section with PM033.

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Autoclave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides with the autoclave for 10 minutes at 110°C. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (1% BSA, 20 mM Hepes, 135 mM NaCl) for 10 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with enzyme conjugated polymer reagent. Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; human tonsil)

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.