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

MONOCLONAL ANTIBODY

Anti-IL-33 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M161-3	4G4	Mouse IgG2a κ	100 µL	1 mg/mL

BACKGROUND: The Interleukin-1 (IL-1) family, such as IL-1α/β and IL-18, has 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 4G4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant protein corresponding to amino acid residues 109 - 266 of mouse IL-33.

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 mouse IL-33 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 2 µg/sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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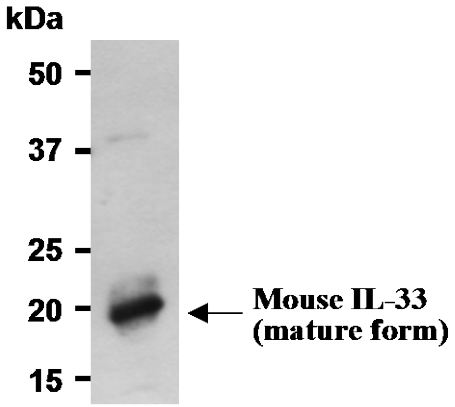
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Sample		Recombinant	
Reactivity on WB	-	+	-

REFERENCES:

1) Ohno, T., *et al.*, *PLoS One* **6**, e18404 (2011)
2) Ohno, T., *et al.*, *J. Immunol.* **183**, 7890-7897 (2009) [WB]
3) Ali, S., *et al.*, *PNAS* **104**, 18660-18665 (2007)
4) Hayakawa, H., *et al.*, *J. Biol. Chem.* **282**, 26369-26380 (2007)
5) Carriere, V., *et al.*, *PNAS* **104**, 282-287 (2007)
6) Sanada, S., *et al.*, *J. Clin. Invest.* **117**, 1538-1549 (2007)
7) Schmitz, J., *et al.*, *Immunity* **23**, 479-490 (2005)
8) Dinarello, C. A., *Immunity* **23**, 461-462 (2005)
9) Baekkevold, E. S., *et al.*, *Am. J. Pathol.* **163**, 69-79 (2003)



Western blot analysis of mouse IL-33 recombinant protein using M161-3.

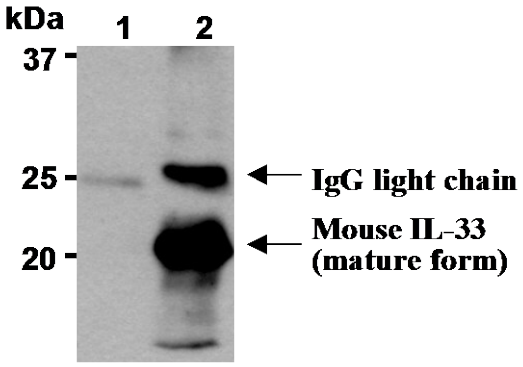
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Mix the recombinant protein with equal volume 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% MeOH). 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 PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) 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.
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.



Immunoprecipitation of recombinant mouse IL-33 protein using mouse IgG2a (1) or M161-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M161-3.*

Immunoprecipitation

1) Add primary antibody as suggest in the **APPLICATIONS** into the sample. 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 IP buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40]. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
3) Wash the beads 3-5 times with the cold IP buffer (centrifuge the tube at 2,500 x g for 10 seconds).
4) 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**.)

RELATED PRODUCTS:

Antibodies

M161-3	Anti-IL-33 (Mouse) mAb (4G4)
M138-3	Anti-IL-33 (Human) mAb (5H1)
M187-3	Anti-IL-33 (Mouse) mAb FG (1F11)
M188-3	Anti-IL-33 (Mouse) mAb FG (2C7)
PM033	Anti-IL-33 (Human) pAb (polyclonal)
D048-3	Anti-IL-18 (Mouse) mAb (93-10C)
D048-3M2	Anti-IL-18 (Mouse) mAb FG (93-10C)
D048-6	Anti-IL-18 (Mouse) mAb-Biotin (93-10C)
D046-3	Anti-IL-18 (Mouse) mAb (39-3F)
D047-3	Anti-IL-18 (Mouse) mAb (74)
D043-3	Anti-IL-18 (Human) mAb (25-2G)
D044-3	Anti-IL-18 (Human) mAb (125-2H)
D044-3M2	Anti-IL-18 (Human) mAb FG (125-2H)
D045-3	Anti-IL-18 (Human) mAb (159-12B)
D045-6	Anti-IL-18 (Human) mAb-Biotin (159-12B)
PM014	Anti-IL-18 (Human) pAb (polyclonal)
M157-3	Anti-IL-18 (Rat) mAb (21A12)
M158-3	Anti-IL-18 (Rat) mAb (91D8)
M163-3	Anti-IL-18 receptor 1 (Mouse) mAb (33A11)
M166-3	Anti-IL-18 receptor 1 (Mouse) mAb (64G4)
M159-3	Anti-IL-18 receptor 1 (Human) mAb (44G6)
D342-3	Anti-IL18R1 (CD218a) (Human) mAb (H44)
D306-3	Anti-IL-18 BP (Mouse) mAb (#36)
D307-3	Anti-IL-18 BP (Mouse) mAb (#31)
D304-3	Anti-IL-18 BP (Human) mAb (#36)
D305-3	Anti-IL-18 BP (Human) mAb (#13)
M156-3	Anti-pro-IL-18 (Human) mAb (43A11)
D065-3	Anti-ST2 (Human) mAb (HB12)
D066-3	Anti-ST2 (Human) mAb (FB9)
D067-3	Anti-ST2 (Human) mAb (2A5)
D067-4	Anti-ST2 (Human) mAb-FITC (2A5)
D067-5	Anti-ST2 (Human) mAb-PE (2A5)

ELISA Kits

5332	Ab-Match ASSEMBLY mouse IL-33 ELISA
7650	Human IL-33 Cytokine domain Detection Kit
7638	Human ST2 ELISA Kit
7625	Mouse IL-18 ELISA Kit
7620	Human IL-18 ELISA Kit

Recombinant Proteins

B006-10	Recombinant Mouse IL-33
B005-10	Recombinant Human IL-33
B002-5	Recombinant Mouse IL-18
B004-5	Recombinant Mouse IL-18 (without BSA)
B001-5	Recombinant Human IL-18
B003-5	Recombinant Human IL-18 (without BSA)