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

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POLYCLONAL ANTIBODY

Anti-IL-18 (Human) pAb

Code No.	Quantity	Form
PM014	100 µL	Purified IgG

BACKGROUND: Interleukin 18 (IL-18) is an 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 rabbit serum using protein A agarose. The rabbit was immunized with human recombinant IL-18.

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human IL-18 (18 kDa) on Western blotting and Immunohistochemistry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HeLa, U937, A431	WR19L	Not tested
Reactivity on WB	+	-	

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APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry; 1:200
Heat treatment for paraffin embedded sections:
Microwave oven, for 20 minutes in 10 mM citrate buffer (pH 6.0)
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

RELATED PRODUCTS:
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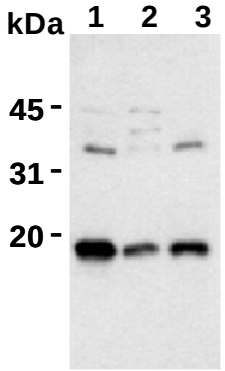
REFERENCES:
1) Hirano, H., *et al.*, *Neuropathology* **32**, 628-637 (2012) [IHC]
2) Komsky, A., *et al.*, *Andrologia* **44**, 1-8 (2012) [IHC]
3) Dao, T., *et al.*, *Cell Immunol.* **173**, 230-235 (1996)
4) Micallef, M., *et al.*, *Eur. J. Immunol.* **26**, 1647-1651 (1996)
5) Ushio, S., *et al. J.*, *Immunol.* **156**, 4274-4279 (1996)
6) Okamura, H., *et al.*, *Nature* **378**, 88-91 (1995)



Western blotting analysis of Human IL-18 expression in HeLa cells (1), U937 cells (2) and A431 cells (3) using PM014.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

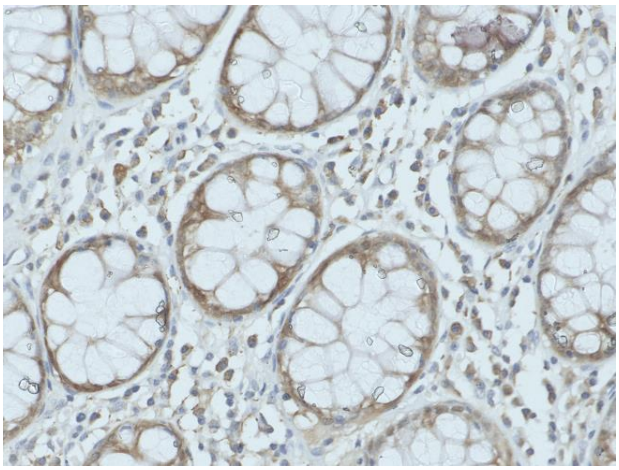
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) 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.
5) 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.
6) 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.
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6).
9) Incubate the membrane with the 1:10,000 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.
10) Wash the membrane with PBS-T (5 minutes x 6).
11) 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.
12) 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; HeLa, U937 and A431)

(a)



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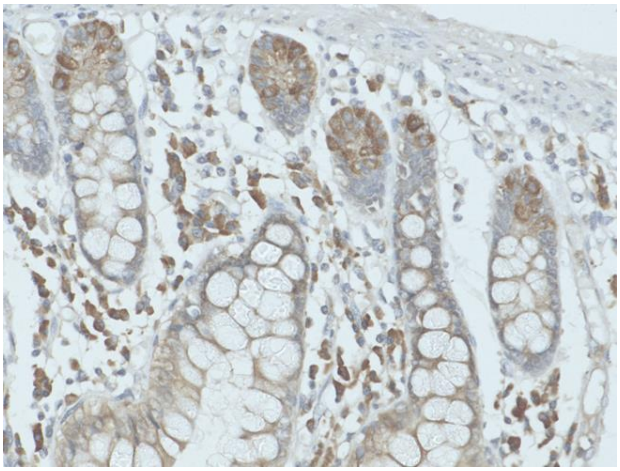

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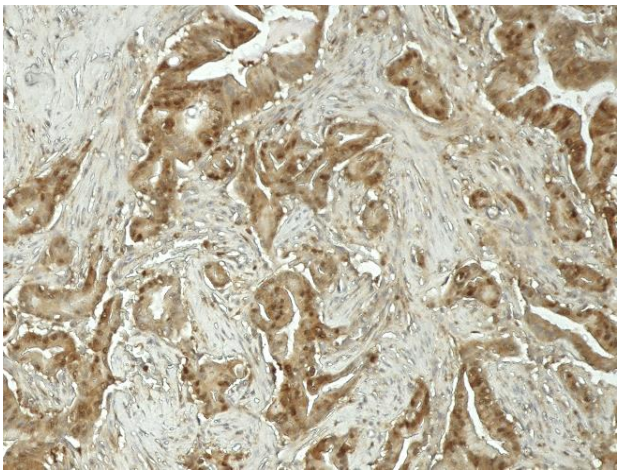

Immunohistochemical detection of human IL-18 on paraffin embedded sections of human colon (a), ileum (b) and pancreatic cancer tissues (c) with PM014.

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 5 minutes each.
2) Wash the slides with Ethanol 3 times for 5 minutes each.
3) Wash the slides with PBS 3 times for 5 minutes each.
4) Remove the slides from PBS and heat-treated with 10 mM Citrate buffer (pH 6.0) for 20 minutes using microwave oven.
5) Let the slides cool down at room temperature in the Citrate buffer.
6) Remove the slides from the Citrate buffer and block endogenous peroxidase with 3% H_2O_2 in PBS for 10 minutes.
7) Wash the slides with PBS twice for 5 minutes each.
8) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes at room temperature (20~25°C) to block non-specific staining. Do not wash.
9) 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** for 1 hour at 4°C. (The concentration of antibody will depend on the conditions.)
10) Wash the slides 3 times in PBS for 5 minutes each.
11) Wipe gently around each section and cover tissues with Histostar™ (Ms + Rb) (MBL, code no. 8460). Incubate for 1 hour at room temperature.
12) Wash the slides 3 times in PBS for 5 minutes each.
13) Visualize by reacting for 5 minutes with Histostar™ DAB (MBL, code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
14) Wash the slides in water for 5 minutes.
15) 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.
16) Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each. Now ready for mounting.

(Positive control for Immunohistochemistry; Human colon, ileum and pancreatic cancer tissue)