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MONOCLONAL ANTIBODY

Anti-Proton Pump (H,K-ATPase α subunit) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D031-3	1H9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Hydrogen-potassium adenosine triphosphatase (H^+, K^+-ATPase) belongs to a family of P-type cation-transporting ATPases that also includes Ca^{2+}-ATPase and Na^+, K^+-ATPase. H^+, K^+-ATPase is responsible for acid secretion into the stomach and catalyzes electroneutral exchange of cytoplasmic hydrogen ions and external potassium ions coupled with ATP hydrolysis. The enzyme is found in gastric parietal cells where it is located in cytoplasmic vesicles or apical plasma membranes of the secretory canaliculus. H^+, K^+-ATPase is a heterodimer consisting of a high molecular weight catalytic α subunit and a smaller but heavily glycosylated β subunit. The α subunit of H^+, K^+-ATPase has 10 transmembrane domains and contains sites for ATP binding and its acylphosphorylation, binding sites of proton pump inhibitors, and sites responsible for ion recognition.

SOURCE: This antibody was purified from ascites fluid using protein A agarose. This hybridoma (clone 1H9) was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Balb/c nu/+ mouse splenocyte with neonatal thymectomy-induced autoimmune gastritis.

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, mouse, rat and bovine H^+, K^+-ATPase α subunit.

APPLICATIONS:

Western blotting; 2-10 µg/mL
Immunoprecipitation; Not tested
It is reported that this monoclonal antibody can be used in Immunoprecipitation in the reference 14).
Immunohistochemistry; 2-10 µg/mL
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Bovine
Reactivity on WB	+	+	+	+

INTENDED USE:

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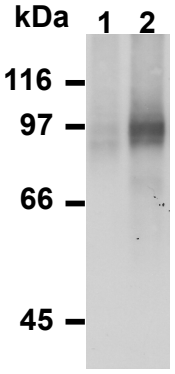
Clone 1H9 is used in these references.



Western blotting analysis of proton pump on mouse stomach using D031-3.
Lane 1: Boiled sample
Lane 2: Non-boiled sample

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

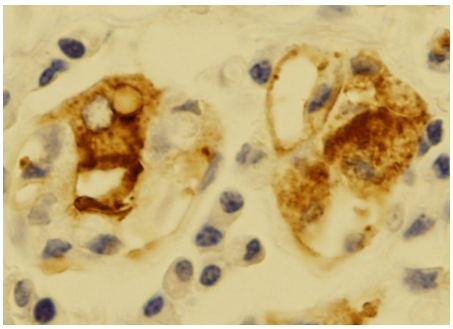
PROTOCOLS:

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. Incubate at room temperature for 1 hour. Don't boil the sample.
4) 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 suggested in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody to be used will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) 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.
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 5 minutes. Develop the film as usual. The condition for exposure and development may vary.



Immunohistochemical detection of proton pump on human stomach paraffin embedded section with D031-3.

(Positive control for Western blotting; Mouse stomach)

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) 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.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl, pH 7.2) for 5 minutes to block non-specific staining. Do not wash.
6) 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**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Histostar™ (Ms + Rb) for Human tissue (MBL, code no. 8460). Incubate for 1 hour at room temperature. Wash as in step 9).
10) Visualize by reacting for 3-10 minutes with Histostar™ DAB Substrate Solution (MBL, code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
11) Wash the slides in water for 5 minutes.
12) Counter stain in hematoxylin for 2 minutes, 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.
13) Now ready for mounting.

(Positive control for Immunohistochemistry; Human stomach)

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