

MONOCLONAL ANTIBODY

Anti-Proton pump/H,K-ATPase β subunit

Code No.	Clone	Subclass	Quantity	Concentration
D032-3	2B6	Mouse IgG1	100 μ g	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 hybridoma (clone 2B6) supernatant using protein A agarose. This hybridoma 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 μ g IgG in 100 μ L 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 β subunit.

APPLICATIONS:

Western blotting; 2-10 μ g/mL for chemiluminescence detection system

Immunoprecipitation; 5-10 μ g/200 - 300 μ L of cell extract

Immunohistochemistry; 2-10 μ g/mL

Immunocytochemistry; Not tested

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	Bovine
Reactivity on WB	+	+	+	+

REFERENCES:

- 1) Kaparakis, M., *et al.*, *Infect. Immun.* **74**, 3519-3529 (2006)
- 2) Vagin, O., *et al.*, *J. Biol. Chem.* **280**, 14741-14754 (2005)
- 3) Rieder, G., *et al.*, *J. Biol. Chem.*, **280**, 4906-4912 (2005)
- 4) Murphy, K., *et al.*, *Int. Immunol.* **15**, 269-277 (2003)
- 5) Laurie, K. L., *et al.*, *J. Immunol.* **169**, 2361-2367 (2002)
- 6) Biondo, M., *et al.*, *J. Immunol.* **166**, 2090-2099 (2001)
- 7) Alderuccio, F., *et al.*, *Int. Immunol.* **12**, 343-352 (2000)
- 8) Lambrecht, N., *et al.*, *J. Biol. Chem.* **275**, 4041-4048 (2000)
- 9) Asano, S., *et al.*, *J. Biol. Chem.* **275**, 8324-8330 (2000)
- 10) Asano, S., *et al.*, *J. Biol. Chem.* **274**, 22257-22265 (1999)
- 11) Stewart, L. A., *et al.*, *Glycobiology.* **9**, 601-606 (1999)
- 12) Jones, C. M., *et al.*, *Gastroenterology* **101**, 287-294 (1991)
- 13) Toh, B., *et al.*, *PNAS.* **87**, 6418-6422 (1990)
- 14) Mori, Y., *et al.*, *Gastroenterology* **97**, 364-375 (1989)

Clone 2B6 is used in these references.

RELATED PRODUCTS:

- D031-3 Proton pump/ H, K-ATPase α subunit (1H9)
D031-3H Proton pump/ H, K-ATPase α subunit (1H9)
D032-3H Proton pump/H, K-ATPase β subunit (2B6)

Clone 1H9 react with human, mouse, rat and bovine H^+ , K^+ -ATPase α subunit.



Western blot analysis of proton pump expression in mouse stomach epithelium using D032-3.

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 cold 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 3 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/cm² 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.
- 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 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.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) 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.
- 10) Wash the membrane with PBS-T (10 minutes x 3 times).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 13) Expose to an X-ray film in a dark room for 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.

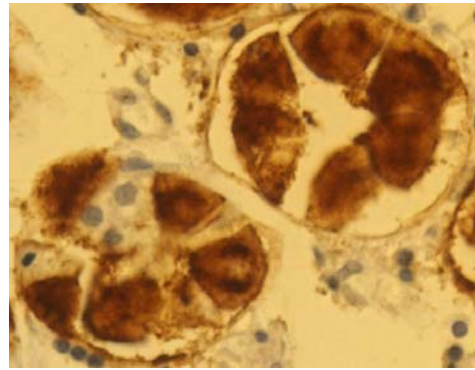
(Positive control for Western blotting; mouse stomach)

Immunoprecipitation

- 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.
- 3) Add primary antibody as suggest in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. 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.

- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) 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**.)



Immunohistochemical detection of proton pump on human stomach paraffin embedded section with D032-3.

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% H₂O₂ 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 Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) 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 PBS containing 1% BSA as suggest 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 Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
- 10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes 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% H₂O₂ 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 controls for Immunohistochemistry; human stomach)