

**MONOCLONAL ANTIBODY**

**Anti-Proton Pump (H,K-ATPase  $\beta$  subunit) mAb**

Code No.	Clone	Subclass	Quantity	Form
<b>D032-3H</b>	<b>2B6</b>	<b>Mouse IgG1</b>	<b>6 mL</b>	<b>Liquid</b>

**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 canalculus.  $H^+$ ,  $K^+$ -ATPase is a heterodimer consisting of a high molecular weight catalytic  $\alpha$  subunit and a smaller but heavily glycosylated  $\beta$  subunit. The  $\alpha$  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:** 6 mL of prediluted antibody containing 0.09%  $NaN_3$ .

\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

**STORAGE:** This antibody solution is stable for 3 years from the date of purchase when stored at 4°C.

**REACTIVITY:** This antibody reacts with human, mouse, rat and bovine  $H^+$ ,  $K^+$ -ATPase  $\beta$  subunit on Immunohistochemistry.

**APPLICATIONS:**

Immunohistochemistry; Ready for use.

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

**INTENDED USE:**

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

**SPECIES CROSS REACTIVITY:**

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

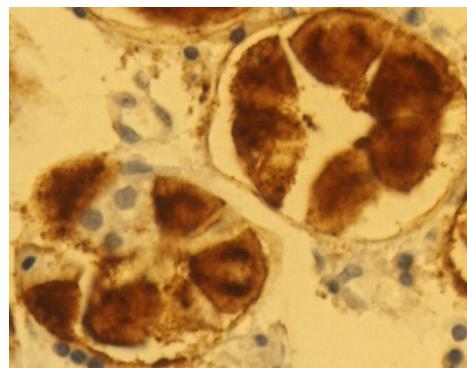
**REFERENCES:**

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Clone 2B6 is used in these references.

**RELATED PRODUCTS:**

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

**PROTOCOL:**

**Immunohistochemical staining for paraffin-embedded sections: SAB method**

- 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 cover each section with 3% H<sub>2</sub>O<sub>2</sub> for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 2 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) 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 one or two drops of Anti-Proton Pump (H,K-ATPase β subunit) mAb (D032-3H) (Ready for use).
- 7) Incubate the sections for 1 hour at room temperature.
- 8) Wash the slides 2 times in PBS for 5 minutes each.
- 9) Wipe gently around each section and cover tissues with Histostart™ (Ms + Rb) for Human tissue (MBL; code no. 8460). Incubate for 30 minutes at room temperature. Wash as in step 8).
- 10) Visualize by reacting for 5 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; stomach)