

POLYCLONAL ANTIBODY

Anti-SLC6A14 (ATB⁰⁺) (Human) pAb

Code No.	Quantity	Form
BMP052	50 µL	Affinity Purified

BACKGROUND: SLC6A14, also known as ATB⁰⁺, is a member of the Na⁺- and Cl⁻- dependent neurotransmitter transporter family, and is highly expressed in the lung, fetal lung, trachea, and salivary gland. SLC6A14 transports both neutral and cationic amino acids, and has about 60% amino acid similarity with the glycine transporters GLYT1 and GLYT2. The blockade of ATB⁰⁺ in cancer cell lines is associated with cell cycle arrest, indicating the potential of ATB⁰⁺ as a drug target in cancer chemotherapy.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC6A14.

FORMULATION: 50 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 can be used to stain endogenous antigen in paraffin embedded human tissues including stomach by Immunohistochemistry. The reactivity has been confirmed by Western blotting, Immunocytochemistry and Flow cytometry to detect the full length of human SLC6A14 transiently expressed in HEK293T cells.

APPLICATIONS:

Western blotting; 1:1,000

Immunoprecipitation; Not tested

Immunohistochemistry; 1:5,000

Heat treatment is necessary for staining paraffin embedded sections.

Autoclave; 125°C for 5 minutes in Tris-EDTA buffer [10mM Tris-HCl, 1mM EDTA, containing 0.05% Tween-20 (pH 9.0)].

Immunocytochemistry; 1:1,000

Flow cytometry; 1:1,000 (final concentration)

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

INTENDED USE:

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

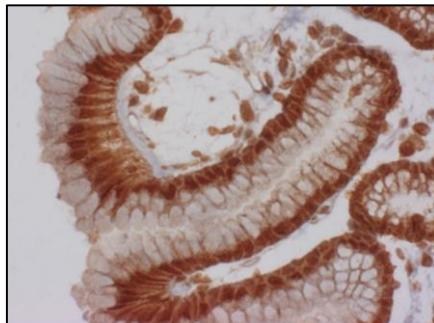
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissue	Stomach	Not tested	Not tested
Reactivity on IHC	+		

REFERENCES:

- 1) Wongthai, P., et al., *Cancer Sci.* **106**, 279-286 (2015) [WB]
- 2) Anderson, C. M. H., et al., *J. Physiol.* **586**, 4061-4067 (2008)
- 3) Karunakaran, S., et al., *Biochem. J.* **414**, 343-355 (2008)
- 4) Sloan, J. L., and Mager, S., *J. Biol. Chem.* **274**, 23740-23745 (1999)

stomach



Immunohistochemical detection of SLC6A14 on paraffin embedded section of human stomach with BMP052. Multi pathological types tissue array (MBL) was used for this application.

PROTOCOLS:

Immunohistochemical staining for paraffin-embedded sections

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.

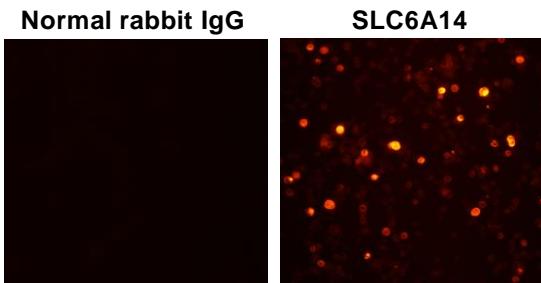
4) Heat treatment

Heat treatment by Autoclave:

Heat the slides immersed in retrieval solution [10mM Tris-HCl, 1mM EDTA, containing 0.05% Tween-20 (pH 9.0)] at 125°C for 5 minutes in pressure boiler. After boiling, the slides should remain in the pressure boiler until the temperature is cooled down to 80°C. Let the immersed slides further cool down at room temperature for 40 minutes.

- 5) Remove the slides from the citrate buffer and cover each section with 0.3% H₂O₂ in PBS for 15 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS containing 0.05% Tween-20 for 5 minutes each.
- 6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (0.5% BSA and 5% Normal goat serum in PBS) for 30 minutes at room temperature to block non-specific staining. Do not wash.
- 7) 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**.
- Note:** It is essential for every laboratory to determine the optional titers of the primary antibody to obtain the best result.
- 8) Incubate the sections for 2 hours at room temperature.
- 9) Wash the slides 3 times in PBS for 5 minutes each.
- 10) Wipe gently around each section and cover tissues with ENVISION/HRP polymer reagent (Thermo Fisher Scientific, code no. K1491). Incubate for 15 minutes at room temperature. Wash as in step 9).
- 11) Visualize by reacting for 5 minutes with DAB substrate solution (Thermo Fisher Scientific, code no. K3465). *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 control for Immunohistochemistry; Human stomach)

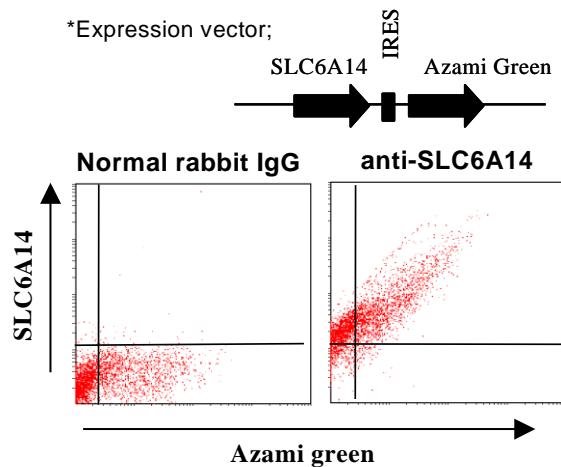


Immunocytochemical detection of SLC6A14 in 293T transiently expressing SLC6A14 with BMP052 (right) or normal rabbit IgG (left).

Immunocytochemistry

- 1) Culture the cells at an appropriate condition on a glass slide. (for example, spread 1 x 10⁴ cells for one slide, then incubate in a CO₂ incubator for one night.)
- 2) Wash the cells 3 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 15 minutes at 4°C.
- 4) Wash the slide twice with PBS containing 0.5% BSA.
- 5) Immerse the slide in PBS containing 0.1% Triton X-100 for 15 minutes at room temperature.

- 6) Wash the cells twice with PBS containing 0.5% BSA, 0.1% Triton X-100.
- 7) Immerse the slide in blocking buffer (0.1% Triton X-100, 0.5% BSA, 5% Normal goat serum, 0.1 mg/mL human IgG in PBS) for 1 hour at room temperature.
- 8) Tip off the washing buffer, add the primary antibody diluted with blocking buffer at a titer as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature (Optimizations of antibody titer or incubation condition are recommended if necessary.).
- 9) Wash the slide 3 times with PBS containing 0.5% BSA, 0.1% Triton X-100.
- 10) Add PE conjugated anti-rabbit IgG antibody diluted with blocking buffer. Incubate in the dark at room temperature for 30 minutes.
- 11) Wash the slide 3 times with PBS containing 0.5% BSA, 0.1% Triton X-100.
- 12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 13) Promptly add mounting medium onto the slide, then put a cover slip on it.



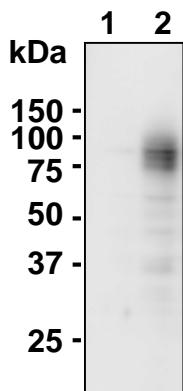
Flow cytometric analysis of intracellular SLC6A14 expression on 293T transiently expressing SLC6A14 and Azami green*. The staining intensity of BMP052 is shown in the vertical axis with Azami Green fluorescence on the horizontal axis.

Flow cytometric analysis

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with PBS containing 2% FCS.
- 2) Resuspend the cells with PBS containing 2% FCS (5x10⁶ cells/mL).
- 3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 100 µL of 4% paraformaldehyde (PFA) in PBS to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
- 5) Wash the cells twice with PBS containing 0.5% BSA.

- 6) Add 100 μ L of PBS containing 0.1% Triton X-100 to the cell pellet after tapping. Mix well, then permeabilize the cells for 15 minutes at room temperature (20~25°C).
- 7) Wash the cells twice with PBS containing 0.5% BSA, 0.1% Triton X-100.
- 8) Add 20 μ L of blocking buffer (PBS containing 0.1% Triton X-100, 0.5% BSA, 5% normal goat serum, 0.1 mg/mL human IgG) to the cell pellet after tapping. Mix well and incubate for 15 minutes at 4°C.
- 9) Add 20 μ L of the primary antibody at a titer as suggested in the **APPLICATIONS** diluted with blocking buffer. Mix well and incubate for 1 hour at room temperature.
- 10) Wash the cells 3 times with PBS containing 2% FCS, 0.1% Triton X-100.
- 11) Add 20 μ L of PE conjugated anti-rabbit IgG at a titer of 1:200 (Beckman Coulter, code no. 732743) diluted blocking buffer. Mix well and incubate in the dark for 30 minutes at room temperature.
- 12) Wash the cells 3 times with PBS containing 0.5% BSA, 0.1% Triton X-100.
- 13) Resuspend the cells with 500 μ L of PBS containing 2% FCS, analyze by a flow cytometer.



Western blotting analysis of SLC6A14 expression in Myc-tagged SLC6A14 transfected 293T (2) and parental cell (1) using BMP052.

- 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% methanol). See the manufacturer's manual for precise transfer procedure.
- 6) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 2 hours at room temperature, or overnight at 4°C.
- 7) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 2% skimmed milk as suggested in the **APPLICATIONS**. (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 3).
- 9) Incubate the membrane with 1:2,000 of Anti-IgG (Rabbit) pAb-HRP (MBL, code no. 458) diluted with 2% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (10 minutes x 3).
- 11) Drain excess buffer on the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 13) Expose and develop the film under usual settings. The condition for exposure and development may vary.

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SDS-PAGE & Western blotting

- 1) Wash cells (approximately 2 x 10⁶ cells) 3 times with PBS and suspend with 100 μ L of cold Lysis buffer [10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1% Sodium deoxycholate, 0.1% SDS] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 fresh tube.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Incubate the samples for 1 hour at 37°C and centrifuge at 10,000 x g for 5 minutes. Transfer the supernatant into a new tube. Load 10 μ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.