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Not for use in diagnostic procedures.

MBL
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POLYCLONAL ANTIBODY

Anti-SLC9A1 (NHE1) (Human) pAb

Code No.	Quantity	Form
BMP023	100 µL	Affinity Purified

BACKGROUND: SLC9A1 (NHE1), a member of the Na^+/H^+ exchanger family, is a ubiquitous membrane protein that regulates intracellular pH and cell volume. Furthermore, SLC9A1 is known to interact with the actin cytoskeleton. Fibroblasts expressing mutated NHE1 that fails to interact with actin-related proteins show impaired organization of focal adhesion points and actin stress fibers, and an irregular cell shape. Polymorphisms of the SLC9A1 gene have been reported in association with essential hypertension.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC9A1.

FORMULATION: 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 can be used to stain endogenous antigen in paraffin embedded human tissues including stomach and kidney by immunohistochemistry. The reactivity of this antibody has been confirmed by Western blotting, Immunocytochemistry and Flow cytometry to detect the full-length of human SLC9A1 transiently expressed in HEK293T cells.

APPLICATIONS:
Western blotting; 1:500
Immunoprecipitation; Not tested
Immunohistochemistry; 1:500
Heat treatment is necessary for staining paraffin embedded sections.
Autoclave; 125°C for 5 minutes in 10 mM citrate buffer containing 0.05% Tween-20 (pH 6.0).
Immunocytochemistry; 1:100
Flow cytometry; 1:100 (final concentration)

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

INTENDED USE:
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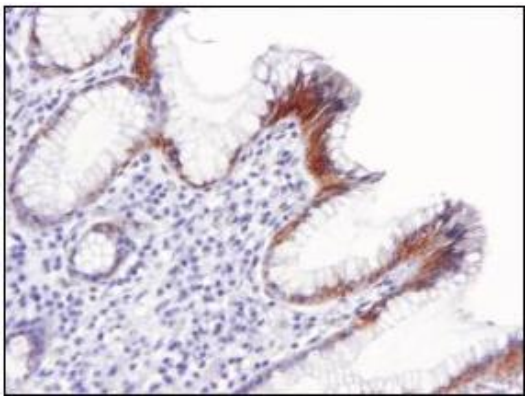
SPECIES CROSS REACTIVITY:

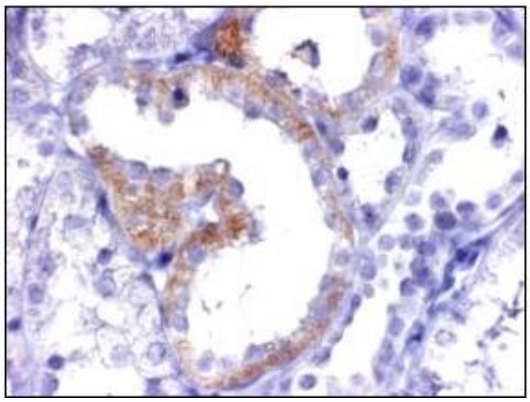
Species	Human	Mouse	Rat
Tissues	stomach, kidney	Not Tested	Not Tested
Reactivity on IHC	+		

REFERENCES:
1) Denker, S. P., *et al.*, *Mol Cell* **6**, 1425-1436 (2000)
2) Dudley, C. R., *et al.*, *Hum Genet* **86**, 79-83 (1990)
3) Sardet, C., *et al.*, *Cell* **56**, 271-280 (1989).

stomach



kidney



Immunohistochemical detection of SLC9A1 on paraffin embedded section of human stomach and kidney with BMP023.

PROTOCOLS:

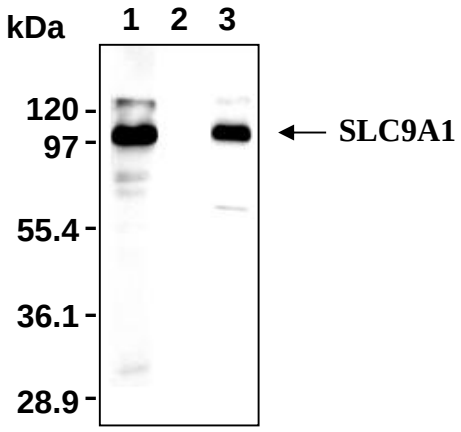
Immunohistochemical staining for paraffin-embedded sections

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 3 times in PBS for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Autoclave:
   Heat the slides immersed in retrieval solution [10 mM citrate buffer containing 0.05% Tween-20 (pH 6.0)] 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 retrieval solution 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.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with 5% FCS 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 PBS containing 5% FCS as suggested in the **APPLICATIONS**.
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 (Agilent). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Visualize by reacting for 5 minutes with DAB substrate solution (Agilent). *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; stomach, kidney)

SDS-PAGE & Western blotting

1) Wash cells (approximately 2 x 10^6 cells) 3 times with PBS and suspend with 100 µL 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 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 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 with primary antibody diluted with 2% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 2 hours 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 3).
9) Incubate the membrane with the 1:2,000 HRP-conjugated anti-rabbit IgG (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, 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 and develop the film as usual. The condition for exposure and development may vary.

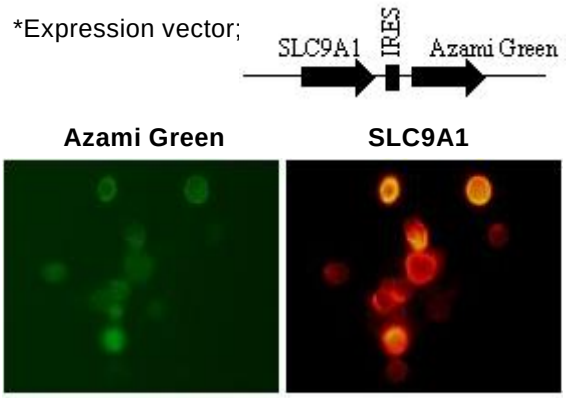


Western blotting analysis of SLC9A1 expression in Myc-tagged SLC9A1 transfected 293T (1, 3) and parental cell (2) using BMP023 (2, 3) or anti-Myc-tag antibody (1, MBL, code no. M047-3).

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at 4°C.

4) Wash the glass slide twice with PBS containing 2% FCS.
5) Immerse the slide in PBS containing 0.1% Triton X-100 for 15 minutes at room temperature.
6) Wash the glass slide twice with PBS containing 2% FCS, 0.1% Triton X-100.
7) Add the primary antibody diluted with PBS containing 2% FCS, 0.1% Triton X-100 as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
8) Wash the glass slide twice with PBS containing 2% FCS, 0.1% Triton X-100.
9) Add 100 µL of 1:200 PE conjugated anti-rabbit IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Wash the glass slide 3 times with PBS containing 2% FCS, 0.1% Triton X-100.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.

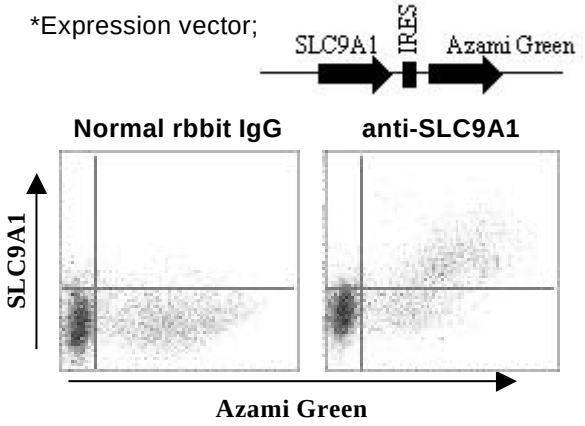


Immunocytochemical detection of SLC9A1 (right) in 293T transiently expressing SLC9A1 and Azami green* with BMP023. Left panel is Azami Green own fluorescence.

Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps after 2).

1) Detach the cells from culture dish by using cell dissociation buffer (Thermo Fisher Scientific, code no. 13151014).
2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% 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.
3) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).
4) 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.
5) Add 100 µL of 4% paraformaldehyde to the cell pellet after tapping. Mix well, then fix the cells for 10 minutes at 4°C.
6) Wash the cells twice with washing buffer.
7) Add 100 µL 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).
8) Wash the cells twice with PBS containing 2% FCS, 0.1% Triton X-100.
9) Add 20 µL of PBS containing 1mg/mL of Human IgG, 2% FCS, 0.1% Triton X-100 to the cell pellet after tapping. Mix well and incubate for 10 minutes at 4°C.
10) Add 20 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with PBS containing 2% FCS, 0.1% Triton X-100. Mix well and incubate for 30 minutes at room temperature.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
12) Add 20 µL of 1:200 PE conjugated anti-rabbit IgG antibody diluted with the washing buffer. Mix well and incubate for 20 minutes at room temperature.
13) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
14) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



Flow cytometric analysis of intracellular SLC9A1 expression on 293T transiently expressing SLC9A1 and Azami green*. *The staining intensity of BMP023 is shown in the vertical axis with Azami Green fluorescence on the horizontal axis.*

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