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POLYCLONAL ANTIBODY

Anti-SLC7A9 ($B^{0/+}$AT) (Human) pAb

Code No.	Quantity	Form
BMP021	50 µL	Affinity Purified

BACKGROUND: SLC7A9, also known as $B^{0/+}$- type amino acid transporter ($B^{0/+}$AT), is a member of the cationic amino acid transporter/glycoprotein-associated family, and it functions as a heterodimer with the amino acid transport protein SLC3A1/rBAT. The SLC7A9 protein is predominantly distributed in the kidney, small intestine, liver, and placenta. Defective mutations in the SLC7A9 and/or SLC3A1 gene cause cystinuria, which is characterized by impaired transport of cystine and dibasic amino acids through the epithelial cells of the renal tubule and gastrointestinal tract; this results in the formation of calculi due to cystine precipitation in the urinary tract, leading to obstruction, infections, and ultimately, renal insufficiency.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC7A9.

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 the small intestine and kidney by Immunohistochemistry. The reactivity has been confirmed by Western blotting, Immunocytochemistry and intracellular Flow cytometry to detect the full length of human SLC7A9 transiently expressed in HEK 293T 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 10 mM citrate buffer containing 0.05% Tween-20 (pH 6.0).
Immunocytochemistry; 1:1,000
Flow cytometry; 1:1,000 (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissues	small intestine, kidney	Not tested	Not tested
Reactivity on IHC	+		

REFERENCES:

1) Eggermann, T., *Hum Genet*. **126**, 330 (2009)
2) Feliubadaló, L., *et al., Hum. Mol. Genet.* **12**, 2097-2108 (2003)
3) Leclerc, D., *et al., Kidney Int*. **62**, 1550-1559 (2002)
4) Colombo, R., *Genomics* **69**, 131-134 (2000)
5) Feliubadalo, L., *et al., Nat. Genet*. **23**, 52-57 (1999)

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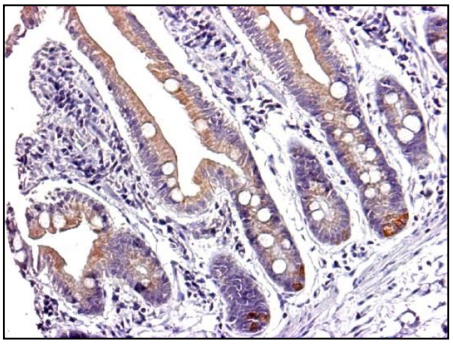
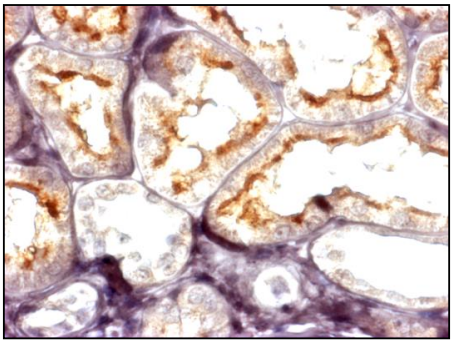
small intestine



kidney



Immunohistochemical detection of SLC7A9 on paraffin embedded section of human small intestine and kidney with BMP021. *Multi pathological types tissue array was used for this application.*

PROTOCOLS:

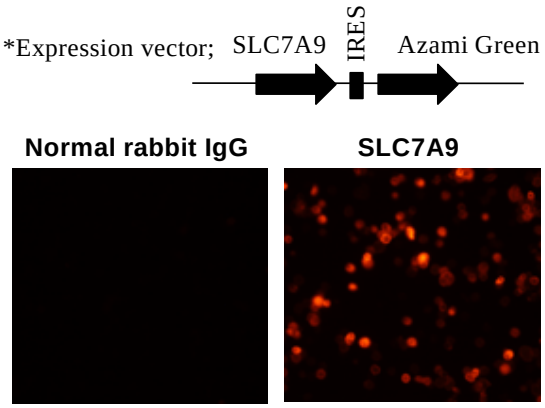
Immunohistochemical staining for paraffin-embedded sections

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 [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 in PBS 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 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 overnight at 4 °C.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Histostar™ (Ms+Rb) (MBL, code no. 8460). Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 10 minutes with DAB substrate solution (MBL, code no. 8469). *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; kidney, small intestine)

Immunocytochemistry

1) Culture the cells at an appropriate condition on a glass slide. (For example, spread $1x10^4$ 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 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, 0.5% BSA for 15 minutes at room temperature.
6) Immerse the slide in blocking buffer (0.1% Triton X-100, 0.5% BSA, 5% Normal goat serum in PBS) for 15 minutes at room temperature.
7) Tip off the blocking buffer, add the primary antibody diluted with blocking buffer at a titer as suggested in the **APPLICATIONS** onto the cells and incubate for 2 hours at room temperature (Optimization of antibody concentration or incubation condition is recommended if necessary).
8) Wash the slide 3 times with PBS containing 0.1% Triton X-100, 0.5% BSA.
9) Add PE conjugated anti-rabbit IgG antibody diluted with blocking buffer. Incubate in the dark for 1 hour at room temperature.
10) Wash the slide 3 times with PBS containing 0.1% Triton X-100, 0.5% BSA.
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.



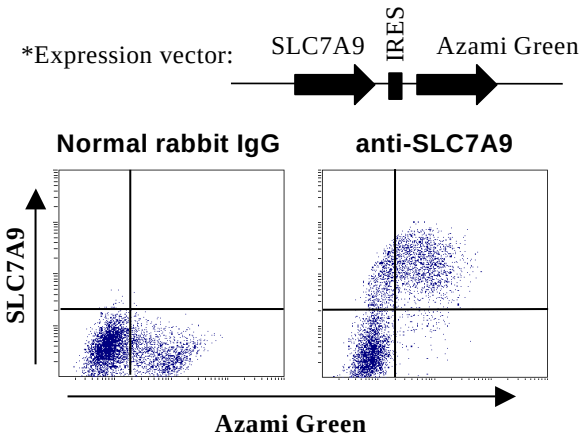
Immunocytochemical detection of SLC7A9 in 293T transiently expressing SLC7A9 using normal rabbit IgG (left) or BMP021 (right).

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 0.5% BSA.
2) Resuspend the cells with PBS containing 0.5% BSA (5 x 10^6 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 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 µL of PBS containing 0.1% Triton X-100, 0.5% BSA to the cell pellet after tapping. Mix well, then permeabilize the cells for 15 minutes at room temperature (20~25°C).

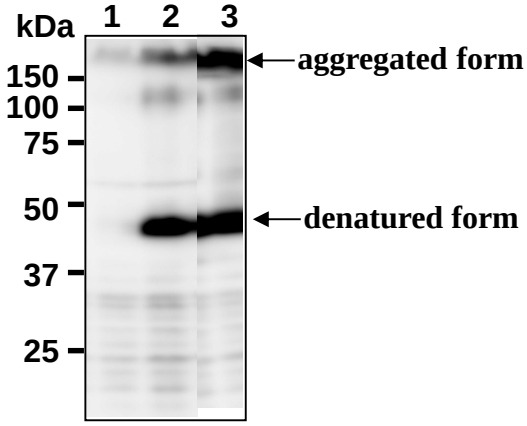
7) Add 50 µL of blocking buffer (PBS containing 0.1% Triton X-100, 0.5% BSA, 5% normal goat serum) to the cell pellet after tapping. Mix well and incubate for 15 minutes at room temperature.
8) Add 50 µL of the primary antibody at a titer as suggested in the **APPLICATIONS** diluted with blocking buffer. Mix well and incubate for 2 hours at room temperature.
9) Wash the cells 3 times with PBS containing 0.1% triton X-100, 0.5% BSA.
10) Add PE conjugated anti-rabbit IgG antibody diluted with blocking buffer. Mix well and incubate in the dark for 1 hour at room temperature.
11) Wash the cells 3 times with PBS containing 0.1% Triton X-100, 0.5% BSA.
12) Resuspend the cells with 500 µL of PBS containing 0.5% BSA, 2 mM EDTA analyzes by a flow cytometer.



Flow cytometric analysis of intracellular SLC7A9 expression in 293T transiently expressing SLC7A9 and Azami green*. *The staining intensity of BMP021 is shown in the vertical axis with Azami Green fluorescence on the horizontal axis.*

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 for 2 hours 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 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, 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.



Western blotting analysis of SLC7A9 expression in Myc-tagged SLC7A9 transfected 293T (2, 3) and parental cell (1) using BMP021 (1, 2) or anti-Myc-tag antibody (3, MBL, code no. M047-3).

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