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Not for use in diagnostic procedures.

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POLYCLONAL ANTIBODY

Anti-SLC7A8 (LAT2) (Human) pAb

Code No.	Quantity	Form
BMP041	50 µL	Affinity Purified

BACKGROUND: SLC7A8, also called L-type amino acid transporter 2 (LAT2), forms a heterodimeric complex with the heavy chain of the cell surface antigen SLC3A2 (4F2hc/CD98). It transports neutral amino acids, most of which are essential amino acids. L-Dopa, which is commonly used to treat Parkinson's disease, can enter the brain as either the SLC7A8 (LAT2)/SLC3A2 complex or as its homologue the SLC7A7 (LAT1)/SLC3A2 complex. Furthermore, SLC7A8 is highly expressed in the epithelial proximal tubule cells in the kidney; this suggests its role in renal reabsorption of neutral amino acids.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC7A8.

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 kidney and pancreas by Immunohistochemistry. The reactivity has been confirmed by Western blotting, Immunocytochemistry, and intracellular Flow cytometry to detect the full length of human SLC7A8 transiently expressed in HEK 293T cells.

APPLICATIONS:

Western blotting; 1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry; 1:1,000
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; 1:200
Flow cytometry; 1:200 (final concentration)

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

INTENDED USE:
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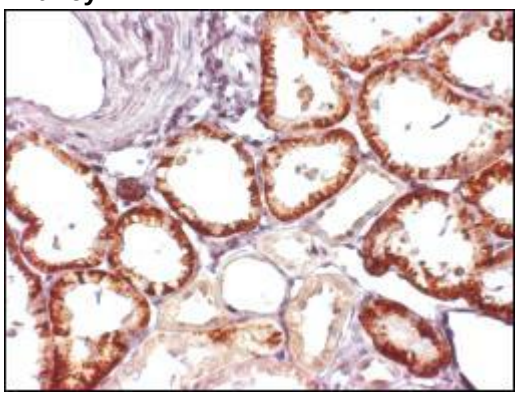
SPECIES CROSS REACTIVITY:

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

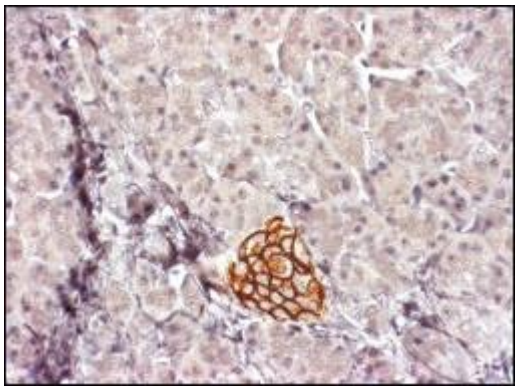
REFERENCES:

1) Torrents, D., *et al.*, *Nature Genet*. **21**, 293-296 (1999)
2) Pineda, M., *et al.*, *J. Biol. Chem*. **274**, 19738-19744 (1999)
3) Torrents, D., *et al.*, *J. Biol. Chem*. **273**, 32437-32445 (1998)

kidney



pancreas



Immunohistochemical detection of SLC7A8 in paraffin embedded section of human kidney and pancreas with BMP041. *Multi pathological types tissue array was used for this application.*

PROTOCOLS:

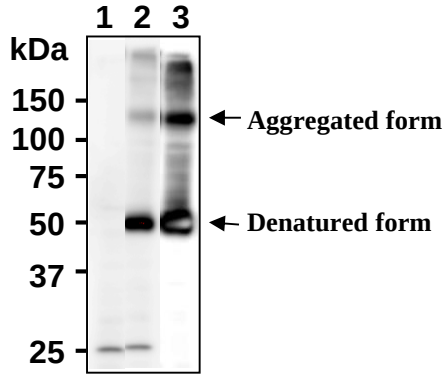
Immunohistochemical staining for paraffin-embedded sections

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 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**.
   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 Histostar™ (Ms+Rb) (MBL, code no. 8460). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Visualize by reacting for 5 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, pancreas)

SDS-PAGE & Western blotting

1) Wash the 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 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) 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 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/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.

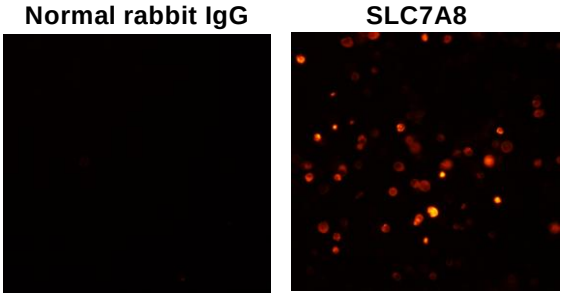


Western blotting analysis of SLC7A8 expression in Myc-tagged SLC7A8 transfected 293T (2, 3) and parental cell (1) using BMP041 (1, 2) or anti-Myc-tag antibody (3, MBL, code no. M047-3).

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 10 minutes at 4°C.

4) Wash the slide twice with PBS containing 0.5% BSA.
5) Immerse the slide in PBS containing 0.5% BSA, 0.1% Triton X-100 for 15 minutes at room temperature.
6) Immerse the slide in blocking buffer (PBS containing 0.1% Triton X-100, 0.5% BSA, 5% normal goat serum and 0.1 mg/mL human IgG) 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 1 hour at room temperature. (Optimizations of antibody titer or incubation condition are recommended if necessary.)
8) Wash the slide 3 times with PBS containing 0.5% BSA, 0.1% Triton X-100.
9) Add PE conjugated anti-rabbit IgG antibody diluted with blocking buffer. Incubate in the dark at room temperature for 30 minutes.
10) Wash the slide 3 times with PBS containing 0.5% BSA, 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.

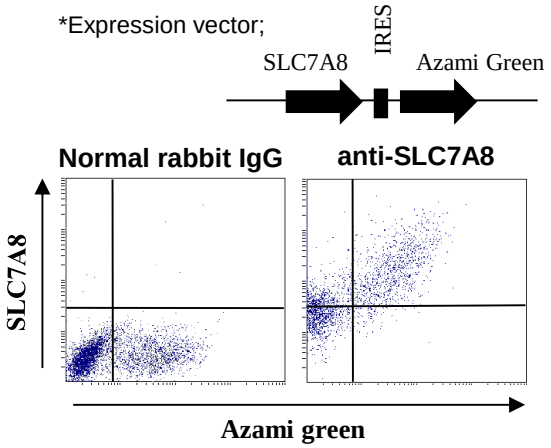


Immunocytochemical detection of SLC7A8 in 293T transiently expressing SLC7A8 with BMP041 (right) or Normal rabbit IgG (left).

Flow cytometric analysis

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

1) Suspend the cells from culture dish with PBS containing 0.5% BSA.
2) Wash the cells twice with PBS containing 0.5% BSA.
3) Resuspend the cells with PBS containing 0.5% BSA (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 10 µL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
6) Wash the cells twice with PBS containing 0.5% BSA.
7) Add 100 µL of PBS containing 0.1% Triton X-100 and 0.5% BSA 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 0.5% BSA, 0.1% Triton X-100.
9) Add 50 µL of blocking buffer (PBS containing 0.1% Triton X-100, 0.5% BSA, 5% normal goat serum and 0.1 mg/mL Human IgG) to the cell pellet after tapping. Mix well and incubate for 15 minutes at 4°C.
10) Add 50 µL 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.
11) Wash the cells 3 times with PBS containing 0.5% BSA, 2 mM EDTA, 0.1% Triton X-100.
12) Add PE conjugated anti-rabbit IgG antibody diluted with the blocking buffer. Mix well and incubate in the dark for 30 minutes at room temperature.
13) Wash the cells 3 times with PBS containing 0.5% BSA, 2 mM EDTA, 0.1% Triton X-100.
14) Resuspend the cells with 500 µL of PBS containing 0.5% BSA, 2 mM EDTA and analyze by a flow cytometer.



Flow cytometric analysis of intracellular SLC7A8 expression in 293T transiently expressing SLC7A8 and Azami green*. *The staining intensity of BMP041 is shown in the vertical axis with Azami Green fluorescence on the horizontal axis.*

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