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

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-SLC1A4/ASCT1

Code No.	Quantity	Form
BMP028	50 µL	Affinity Purified

BACKGROUND: SLC1A4 (ASCT1), a member of the neutral amino acid transporter family, is expressed in most tissues. Originally found in the brain, it mediates Na^+-dependent exchange of small neutral amino acids such as alanine, serine, cysteine, and threonine. The SLC1A4 gene is located on chromosome 2p13-p14, a region related to schizophrenia (SCZ) and bipolar disorder (BD). Polymorphisms in the SLC1A4 gene associated with SCZ or BD, however, have not been found yet.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC1A4.

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 -20ºC.

REACTIVITY: This antibody can be used to stain endogenous antigen in paraffin embedded human tissues including kidney by Immunohistochemistry. The reactivity has been confirmed by Western blotting, Immunocytochemistry and Flowcytometry to detect the full length of human SLC1A4 transiently expressed in HEK 293T cells.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; 1:1,000
Heat treatment is necessary for staining paraffin embedded sections.
Autoclave; 125ºC 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:

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

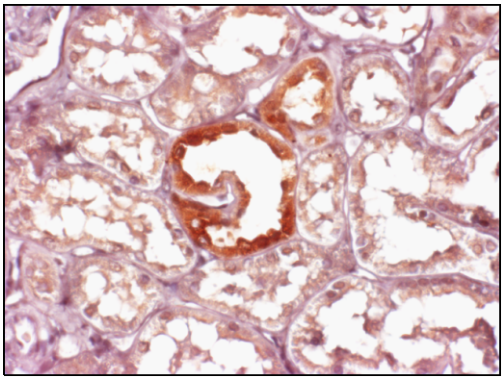
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissue	kidney	Not Tested	Not Tested
Reactivity on IHC	+		

REFERENCES:

1) Deng, X, *et al., BMC Psychiatry* **8**, 58-66 (2008)
2) Skowronek, M. H., *et al., Psychiatr Genet.* **16**, 233-234 (2006)
3) Hofmann, K., *et al., Genomics* **24**, 20-26 (1994)

kidney



Immunohistochemical detection of SLC1A4 on paraffin embedded section of human kidney with BMP028. *Multi pathological types tissue array (MBL) was used for this application.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

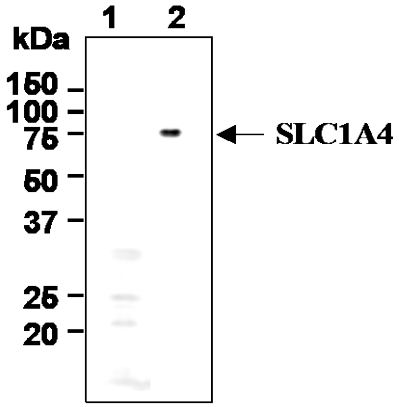
Immunohistochemical staining for paraffin-embedded sections

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 citrate buffer 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 (DAKO; 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 (DAKO; 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.

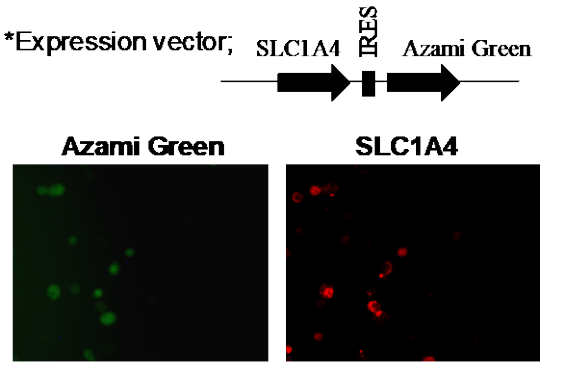
(Positive control for Immunohistochemistry; human kidney)



Western blot analysis of SLC1A4 expression in parental cell (1) and Myc-tagged SLC1A4 transfected 293T (2) using BMP028.

SDS-PAGE & Western Blotting

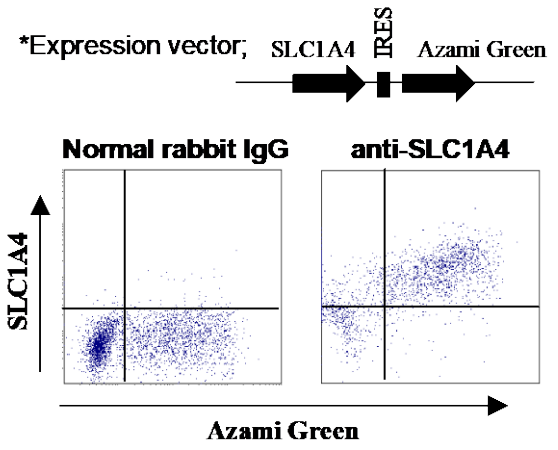
1) Wash cells (approximately 2 x 10^6 cells) 3 times with PBS and suspend with 100 µL of cold Lysis buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% Sodium deoxycholate, 0.1% SDS) containing appropriate protease inhibitors. Incubate it at 4ºC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4ºC 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 37ºC 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% MeOH). 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 4ºC.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 2% skimmed milk 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 times).
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 times).
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.



Immunocytochemical detection of SLC1A4 (right) in 293T transiently expressing SLC1A4 and Azami green* with BMP028. Left panel is Azami Green own fluorescence.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread $1x10^4$ cells for one slide, then incubate in a CO_2 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 for 10 minutes at 4ºC.
4) Wash the glass slide 2 times 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 2 times 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 2 times with PBS containing 2% FCS, 0.1% Triton X-100.
9) Add 100 µL of 1:200 PE conjugated anti-rabbit IgG (Beckman Coulter; code no. 732743) 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.



Flow cytometric analysis of intracellular SLC1A4 expression on 293T transiently expressing SLC1A4 and Azami green*. *The staining intensity of BMP028 is shown in the vertical axis with Azami Green fluorescence on the horizontal axis.*

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 (Invitrogen; code no. 13151-014).
2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
3) Resuspend the cells with washing buffer ($5x10^6$ 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 4ºC.
6) Wash the cells 2 times 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 (20~25ºC).
8) Wash the cells 2 times 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 4ºC.
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 (Beckman Coulter; code no. 732743) 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.

RELATED PRODUCTS:

Please visit our web site https://ruo.mbl.co.jp/