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

POLYCLONAL ANTIBODY

Anti-SLC1A2/EAAT2

Code No.	Quantity	Form
BMP069	100 µL	Affinity Purified

BACKGROUND: Members of the solute carrier family 1 are classified as high-affinity glutamate and neutral amino acid transporters. These are also referred to as excitatory amino acid transporters, which regulate glutamatergic signal transmission by clearing excess glutamate after synaptic release in the central nervous system. SLC1A2/EAAT2 is predominantly located on the astroglia in the brain and is believed to act as a glial-specific glutamate transporter. Lethal seizures and increased susceptibility to acute cortical injury have been observed in mice with disrupted *slc1a2* gene. In humans, a severe decrease in the expression levels of SLC1A2/EAAT2 is noted in the brain of patients with amyotrophic lateral sclerosis.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC1A2.

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 the cerebrum by Immunohistochemistry. The reactivity has been confirmed by Immunocytochemistry and intracellular Flow cytometry to detect the full length of human SLC1A2 transiently expressed in HEK 293T cells.

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; Not tested
Immunohistochemistry (for paraffin embedded section); 1:2,500
Heat treatment is necessary.
Autoclave; 125°C for 5 minutes in 10 mM citrate buffer containing 0.05% Tween-20 (pH 6.0).
Immunocytochemistry; 1:500
Flow cytometry; 1:500 (final concentration)

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

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

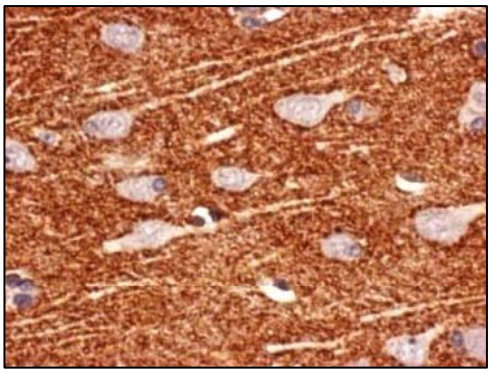
REFERENCES:

1) Mallolas, J., *et al., J. Exp. Med*. **203**, 711-717 (2006)
2) Su, Z., *et al., PNAS* **100**, 1955-1960 (2003)
3) Trotti, D., *et al., J. Biol. Chem*. **276**, 576-582 (2001)
4) Tanaka, K., *et al., Science* **276**, 1699-1702 (1997)
5) Shashidharan, P., *et al., Biochim. Biophys. Acta.* **1191**, 393-396 (1994)
6) Rothstein, J. D., *et al., Neuron* **13**, 713-725 (1994)
7) Krishnan, S. N., *et al., Soc. Neurosci. Abstr*. **19**, 219 (1993)

SPECIES CROSS REACTIVITY:

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

cerebrum



Immunohistochemical detection of SLC1A2 on paraffin embedded section of human cerebrum with BMP069. *Multi pathological types tissue array (MBL) was used for this application.*

PROTOCOLS:

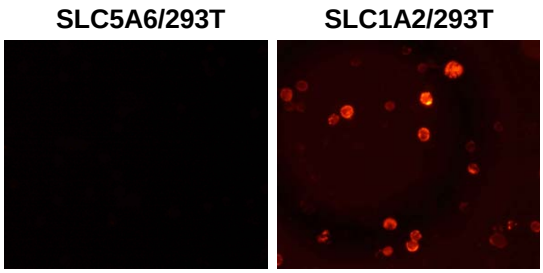
Immunohistochemical staining for paraffin-embedded sections

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 [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 over night 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.

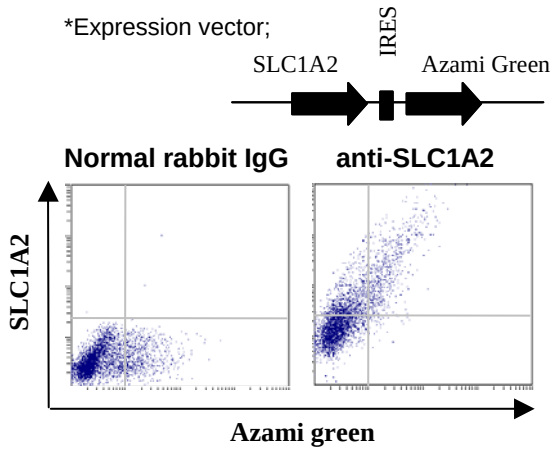
(Positive control for Immunohistochemistry; cerebrum)



Immunocytochemical detection of SLC1A2 in 293T transiently expressing SLC5A6 (left) or SLC1A2 (right) with BMP069.

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 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 2 times 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 washing 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 (Optimizations of antibody titer or incubation condition are recommended if necessary.)
8) Wash the slide 3 times with PBS containing 0.1% Triton X-100, 0.5% BSA.
9) Add 50 µL of PE conjugated anti-rabbit IgG (Beckman Coulter; code no. 732743) at a titer of 1:200 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.



Flow cytometric analysis of intracellular SLC1A2 expression on 293T transiently expressing SLC1A2 and Azami green*. *The staining intensity of BMP069 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 0.5% BSA.
2) Resuspend the cells with PBS containing 0.5% BSA (5x10^6 cells/mL).
3) Add 100 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 2 times 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 50 µL of PE conjugated anti-rabbit IgG at a titer of 1:200 (Beckman Coulter; code no. 732743) 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 analyze by a flow cytometer.

RELATED PRODUCTS:

BMP010	anti-SLC1A1/EAAC1/EAAT3 (polyclonal)
BMP069	anti-SLC1A2/EAAT2 (polyclonal)
BMP009	anti-SLC1A3/GLAST1/EAAT1 (polyclonal)
BMP028	anti-SLC1A4/ASCT1 (polyclonal)
BMP058	anti-SLC1A6/EAAT4 (polyclonal)
BMP024	anti-SLC1A7/EAAT5 (polyclonal)