

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



Anti-SLC6A9/GlyT1

CODE No. BMP091

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from rabbit serum
FORMURATION 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.

APPLICATIONS-CONFIRMED

Western blotting 1:250 for chemiluminescence detection system

Immunohistochemistry 1:1,000

Heat treatment for paraffin embedded section:

Autoclave; 125 °C for 5 minutes in 10 mM Tris-HCl (pH 9.0) containing 1 mM EDTA, 0.05% Tween-20

Flow cytometry 1:500

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	Transfectant	Not Tested	Not Tested	Not Tested
Reactivity	+			

Entrez Gene ID 6536 (Human)

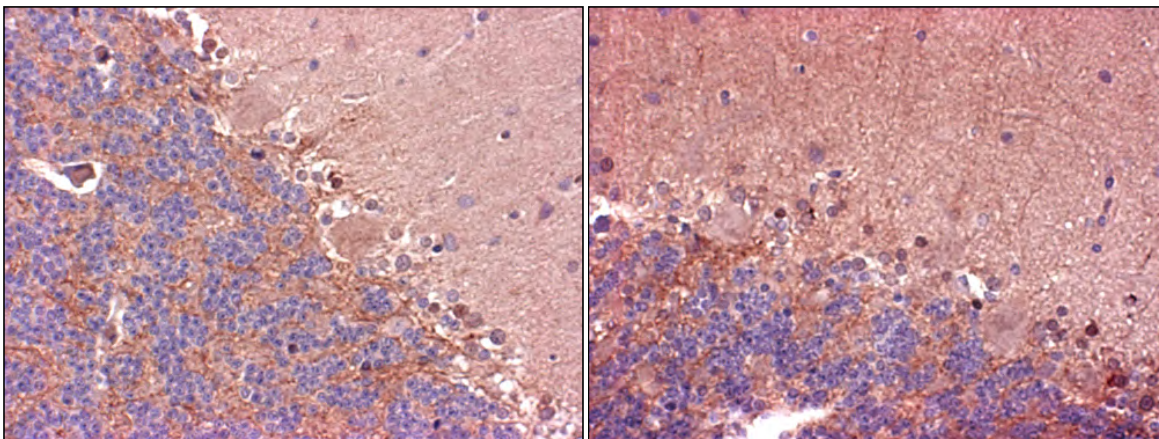
REFERENCES

- 1) Tsai, G., *et al.*, *PNAS* **101**, 8485-8490 (2004)
- 2) Gomeza, J., *et al.*, *Neuron* **40**, 785-796 (2003)
- 3) Kim, K. M., *et al.*, *Molec.Pharmacol.* **45**, 608-617 (1994)
- 4) Borowsky, B., *et al.*, *Neuron* **10**, 851-863 (1993)

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Immunohistochemistry for formalin fixed paraffin-embedded section

- 1) Deparaffinize the sections with Xylene 3 times for 5 minutes each.
- 2) Wash the slides with Ethanol (100%, 95%, 90%, 80%, 70%) for 3 minutes each.
- 3) Wash the slides with PBS 3 times for 5 minutes each.
- 4) Heat treatment
Heat treatment by Autoclave:
Heat the slides immersed in retrieval solution [10 mM Tris-HCl (pH 9.0) containing 1 mM EDTA, 0.05% Tween-20] 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% H₂O₂ in PBS for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 2 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, 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 suggest in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.) Incubate the sections for overnight at 4°C.
- 8) Wash the slides 3 times in PBS for 5 minutes each.
- 9) Wipe gently around each section and cover tissues with Histostar™ (mouse + rabbit) (MBL; code no. 8460). Incubate for 1 hour at room temperature. Wash as in step 8).
- 10) Visualize by reacting for 10 minutes with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 11) Wash the slides in water for 5 minutes.
- 12) 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.
- 13) Now ready for mounting.

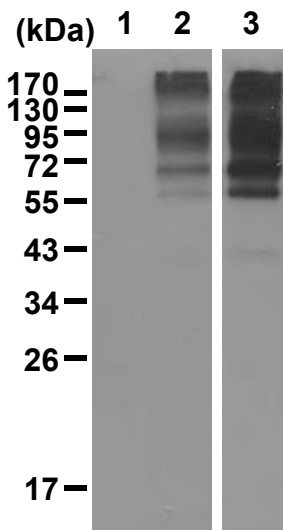


Immunohistochemical detection of SLC6A9

Cerebella from different donors
Immunohistochemical staining with BMP091
Normal human tissue array (MBL) was used for this application.

SDS-PAGE & Western blotting

- 1) Wash 2×10^6 cells 3 times with PBS and suspends them in 100 μ L of Extraction buffer (10 mM Tris-HCl (pH7.5), 150 mM NaCl, 1% Triton X-100, 1% Sodium deoxycholate, 0.1% SDS), then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add equal volume of Laemmli's sample buffer, and then incubate the samples for 1 hour at 37°C and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS (5 minutes x 3 times).
- 8) Incubate the membrane with the 1:5,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 9) Wash the membrane with PBS (5 minutes x 3 times).
- 10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.



Western blot analysis of Myc-tagged SLC6A9

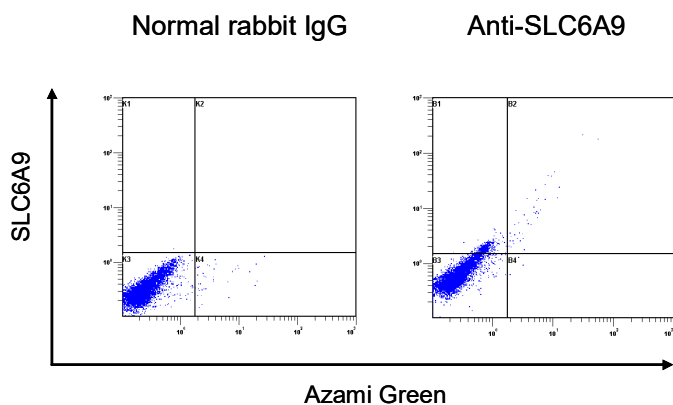
Lane 1: Parental cell (293T)
Lane 2 and 3: Myc-tagged SLC6A9/293T

Immunoblot
Lane 1 and 2: BMP091
Lane 3: anti-Myc-tag (MBL; code no. M047-3)

Flow cytometric analysis for adherent cells

- 1) Detach the cells from culture dish.
- 2) Wash the cells 3 times with 1 mL of washing buffer [PBS containing 0.5% BSA].
- 3) Resuspend the cells with washing buffer (5×10^6 cells/mL).
- 4) Add 100 μ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 5) Add 100 μ L of 4% paraformaldehyde (PFA) in PBS to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
- 6) Wash the cells 2 times with 1 mL of washing buffer.
- 7) Add 100 μ L 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.
- 8) Wash the cells 2 times with 1 mL of washing buffer.
- 9) Add 50 μ L 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 10 minutes at room temperature.
- 10) Add 50 μ L of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with the blocking buffer. Mix well and incubate for 2 hours at room temperature.
- 11) Add 1 mL of the PBS containing 0.1% Triton X-100, 0.5% BSA followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration. Repeat another wash twice more.
- 12) Add 50 μ L of 1:200 PE conjugated anti-rabbit IgG (MBL; code no. 732743) diluted with the blocking buffer. Mix well and incubate in the dark for 1 hour at room temperature.
- 13) Add 1 mL of the PBS containing 0.1% Triton X-100, 0.5% BSA followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration. Repeat another wash twice more.
- 14) Resuspend the cells with 500 μ L of the PBS containing 0.5% BSA, 2 mM EDTA and analyze by a flow cytometer.

*Expression vector



Flow cytometric detection of intracellular SLC6A9

Cells: SLC6A9-IRES-Azami Green/293T (Transient)