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

POLYCLONAL ANTIBODY

# Anti-SLC6A6 (TAUT) (Human) pAb

| Code No. | Quantity | Form |
|---|---|---|
| BMP046 | 50 µL | Affinity Purified |

**BACKGROUND:** SLC6A6, also known as TAUT, belongs to the neurotransmitter transporter family and transports taurine in a $Na^+$- and $Cl^-$-dependent manner. Taurine is involved in many important physiological processes, including antioxidation, osmolarity regulation, and proper maintenance of the functions of the liver and skeletal muscles. Since fetuses and neonates cannot independently synthesize taurine, they depend on taurine supplied through the placenta and breast milk of their mothers for their development. According to a recent report, rat SLC6A6 also appears to transport another neurotransmitter, namely, GABA, across the inner blood-retinal barrier.

**SOURCE:** This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC6A6.

**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 cerebral cortex by Immunohistochemistry. The reactivity has been confirmed by Immunocytochemistry, and intracellular Flow cytometry to detect the full length of human SLC6A6 transiently expressed in HEK 293T cells.

**APPLICATIONS:**

Western blotting; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1).
Immunoprecipitation; Not tested
Immunohistochemistry; 1:5,000
Heat treatment is necessary for staining paraffin embedded sections.
Autoclave; 125°C for 5 minutes in Tris-EDTA buffer [10mM Tris-HCl, 1mM EDTA, containing 0.05% Tween-20 (pH 9.0)].
Immunocytochemistry; 1:200
Flow cytometry; 1:200 (final concentration)

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

**SPECIES CROSS REACTIVITY:**

| Species | Human | Mouse | Rat | Others* |
|---|---|---|---|---|
| Tissue | Cerebral cortex | Not tsted | Not tested | Not tested |
| Reactivity on IHC | + | | | |

*Reactivity of this antibody to other species is not confirmed in our laboratory. However, it is reported that this antibody reacts with Japanese eel[1)].

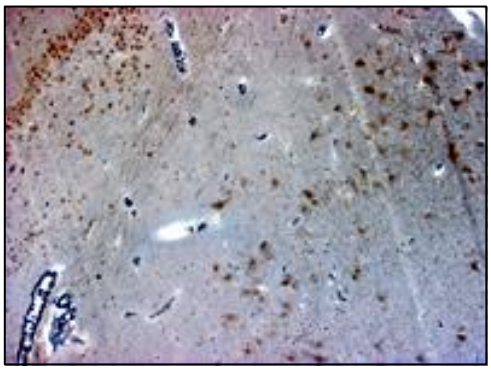
**INTENDED USE:**

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

**REFERENCES:**

1) Higuchi, M., *et al.*, *Amino Acids.* 43, 2359-2369 (2012) [WB, IHC]
2) Tomi, M., *et al.*, *Biochim. Biophys. Acta*. **1778**, 2138-2142 (2008)
3) Ito, T., *et al.*, *J. Mol. Cell Cardiol*. **44**, 927-937 (2008)
4) Warskulat, U., *et al.*, *Methods Enzymol.* **428**, 439-458 (2007)
5) Ramamoorthy, S., *et al.*, *Biochem. J.* **300**, 893-900 (1994)
6) Uchida, S., *et al.*, *PNAS.* **89**, 8230-8234 (1992)

**cerebral cortex**



***Immunohistochemical detection of SLC6A6 on paraffin embedded section of human cerebral cortex with BMP046.*** *Multi pathological types tissue array (MBL) was used for this application.*

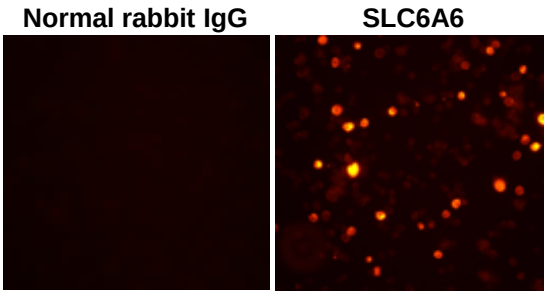
**PROTOCOLS:**

**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 [10mM Tris-HCl, 1mM EDTA, containing 0.05% Tween-20 (pH 9.0)] 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 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 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.

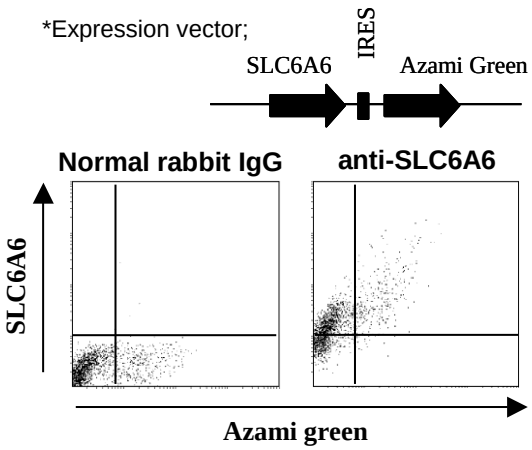
(Positive control for Immunohistochemistry; Cerebral cortex)




***Immunocytochemical detection of SLC6A6 in 293T transiently expressing SLC6A6 with BMP046 (right) or normal rabbit IgG (left).***

## 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 4% paraformaldehyde (PFA) in PBS for 10 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 for 15 minutes at room temperature.
6) Wash the slide 2 times with PBS containing 0.5% BSA, 0.1% Triton X-100.
7) Tip off the washing buffer, add the primary antibody diluted with PBS containing 0.5% BSA, 0.1% Triton X-100, 5% Normal goat serum at a titer as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes 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% FCS, 0.1% Triton X-100.
9) Add 100 µL of PE conjugated anti-rabbit IgG (Beckman Coulter; code no. 732743) at a titer of 1:200 diluted with PBS containing 1% Normal goat serum, 0.1% Triton X-100, 0.5% BSA. 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.




***Flow cytometric analysis of intracellular SLC6A6 expression on 293T transiently expressing SLC6A6 and Azami green*.*** *The staining intensity of BMP046 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 2% FCS.

2) Resuspend the cells with PBS containing 2% FCS ($5x10^6$ 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 (PFA) in PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 minutes at 4°C.
5) Wash the cells 2 times with PBS containing 2% FCS.
6) 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 (20~25°C).
7) Wash the cells 2 times with PBS containing 2% FCS, 0.1% Triton X-100.
8) Add 20 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 4°C.
9) Add 20 µL of the primary antibody at a titer as suggested in the **APPLICATIONS** diluted with blocking buffer. Mix well and incubate for 30 minutes at room temperature.
10) Wash the cells 3 times with PBS containing 2% FCS, 0.1% triton X-100.
11) Add 20 µL of PE conjugated anti-rabbit IgG at a titer of 1:200 (Beckman Coulter; code no. 732743) diluted with PBS containing 1% Normal goat serum, 0.1% Triton X-100, 0.5% BSA. Mix well and incubate in the dark for 20 minutes at room temperature.
12) Wash the cells 3 times with PBS containing 0.5% BSA, 0.1% triton X-100.
13) Resuspend the cells with 500 µL of PBS containing 2% FCS, analyze by a flow cytometer.

## RELATED PRODUCTS:

BMP029 Anti-SLC6A2 (NET) (Human) pAb
BMP015 Anti-SLC6A3 (DAT1) (Human) pAb
BMP045 Anti-SLC6A4 (SERT) (Human) pAb
BMP016 Anti-SLC6A7 (PROT) (Human) pAb
BMP047 Anti-SLC6A8 (CRTR) (Human) pAb
BMP091 Anti-SLC6A9 (GlyT1) (Human) pAb
BMP038 Anti-SLC6A12 (BGT-1) (Human) pAb
BMP051 Anti-SLC6A13 (GAT2) (Human) pAb
BMP052 Anti-SLC6A14 ($ATB^{0+}$) (Human) pAb
BMP050 Anti-SLC6A15 (SBAT1) (Human) pAb
BMP053 Anti-SLC6A19 ($B^0AT1$) (Human) pAb