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

POLYCLONAL ANTIBODY

Anti-SLC6A4/SERT

Code No.	Quantity	Form
BMP045	50 µL	Affinity Purified

BACKGROUND: SLC6A4, also known as a serotonin transporter (SERT), belongs to the Na^+- and Cl^--dependent neurotransmitter transporter family and transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons. This activity of SLC6A4 terminates the synaptic actions of serotonin, and the presynaptic neurons release it once again into the neurotransmitter pool. A repeat length polymorphism in the *SLC6A4* gene promoter has been shown to affect the rate of serotonin uptake and may thus be associated with the sudden infant death syndrome, aggressive behavior in Alzheimer disease patients, and the susceptibility of people experiencing emotional trauma to depression.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC6A4.

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 Western blotting to detect the full length of human SLC6A4 transiently expressed in HEK 293T cells.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
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; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissues	cerebral cortex	Not Tested	Not Tested
Reactivity on IHC	+		

INTENDED USE:

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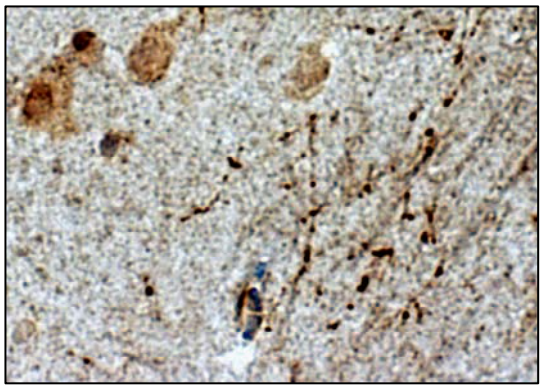
REFERENCES:

1) Lesch, K. P., *et al.*, *Science* **274**, 1527-1530 (1996)
2) Ramamoorthy, S., *et al.*, *PNAS* **90**, 2542-2546 (1993)

cerebral cortex



Immunohistochemical detection of SLC6A4 on paraffin embedded section of human cerebral cortex with BMP045. *Multi pathological types tissue array (MBL) was used for this application.*

PROTOCOLS:

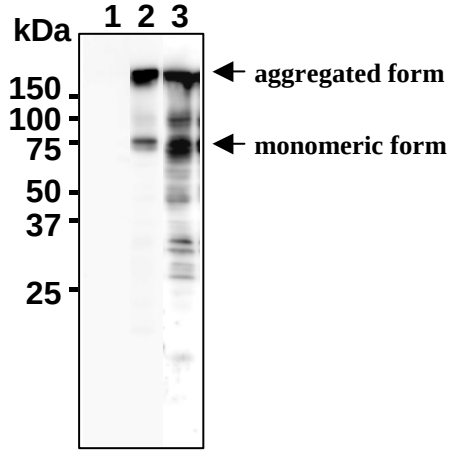
Immunohistochemical staining for paraffin-embedded sections

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 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 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 1 hour 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)



Western blot analysis of SLC6A4 expression in Myc-tagged SLC6A4 transfected 293T (2, 3) and parental cell (1) using BMP045 (1, 2) or anti-Myc-tag antibody (3, MBL; code no. M047-3).

SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 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 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 4°C.
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 condition.)
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.

RELATED PRODUCTS:

BMP029	anti-SLC6A2/NET (polyclonal)
BMP015	anti-SLC6A3/DAT1 (polyclonal)
BMP045	anti-SLC6A4/SERT (polyclonal)
BMP046	anti-SLC6A6/TAUT (polyclonal)
BMP016	anti-SLC6A7/PROT (polyclonal)
BMP047	anti-SLC6A8/CRTR (polyclonal)
BMP038	anti-SLC6A12/BGT-1 (polyclonal)
BMP051	anti-SLC6A13/GAT2 (polyclonal)
BMP052	anti-SLC6A14/ATB^{0+} (polyclonal)
BMP050	anti-SLC6A15/SBAT1 (polyclonal)
BMP053	anti-SLC6A19/B^0AT1 (polyclonal)