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MBL A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-SLC7A5 (LAT1) (Human) pAb

Code No.	Quantity	Form
BMP011	100 µL	Affinity Purified

BACKGROUND: SLC7A5, L-type amino acid transporter 1 (LAT1), forms as a heterodimeric complex with the heavy chain of the cell surface antigen 4F2 (4F2hc/CD98). It transports neutral amino acids, most of which are essential amino acids across blood brain barrier. L-dopa, which is commonly used to treat Parkinson's disease, also enters the brain the LAT1/CD98 complex. In addition, tumors with increased expression of SLC7A5 have been reported. Increased uptake of amino acids through SLC7A5 may facilitate tumor cell growth and survival.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC7A5.

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 brain by immunohistochemistry. The reactivity of this antibody has been confirmed by Western blotting to detect the full-length of human SLC7A5 transiently expressed in HEK 293T cells.

APPLICATIONS:

Western blotting; 1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry; 1:1,000
Heat treatment is necessary for staining paraffin embedded sections.
Autoclave; 125°C for 5 minutes in 10 mM citrate buffer containing 0.05% Tween-20 (pH 6.0).
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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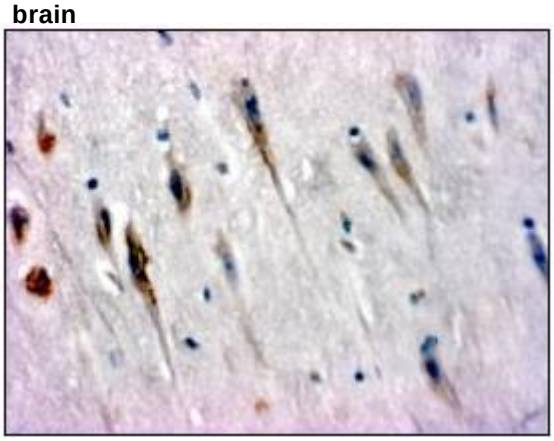
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissue	Brain	Not tested	Not tested
Reactivity on IHC	+		

REFERENCES:

1) Barel, M., *et al.*, *Cell Microbiol.* **14**, 1769-1783 (2012) [WB]
2) William, A., *et al.*, *J. Biol. Chem.* **276**, 16877-16884 (2001)
3) Kageyama, T., *et al.*, *Brain Res.* **879**, 115-121 (2000)
4) Mastroberardino, L., *et al.*, *Nature* **395**, 288-291 (1998)

brain



Immunohistochemical detection of SLC7A5 on paraffin embedded section of human brain with BMP011. *Multi pathological types tissue array (MBL) was used for this application.*

PROTOCOLS:

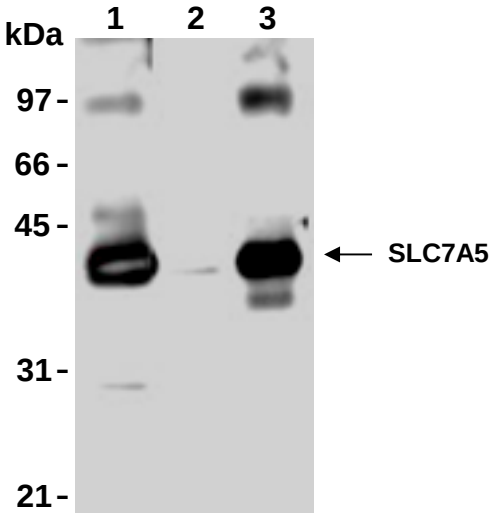
Immunohistochemical staining for paraffin-embedded sections

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 3 times in PBS for 3-5 minutes each.
4) 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 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 brain)



Western blotting analysis of SLC7A5 expression in Myc-tagged SLC7A5 transfected 293T (1, 3) and parental cell (2) using anti-Myc-tag antibody (1, MBL; code no. M047-3) or BMP011 (2, 3).

SDS-PAGE & Western blotting

1) Wash cells (approximately 2 x 10^6 cells) 3 times with PBS and resuspend them in 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 protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 fresh tube.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Incubate the samples for 1 hour at 60°C and centrifuge at 10,000 x g for 5 minutes. Transfer the supernatant into a new tube. Load 10 µL of the 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% methanol). 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 for 2 hours at room temperature with primary antibody diluted with 2% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (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).
9) Incubate the membrane with 1:2,000 of Anti-IgG (Rabbit) pAb-HRP (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).
11) Drain excess buffer on the membrane, and incubate the with an 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 the membrane onto an X-ray film in a dark room for 1 minute.
14) Develop the film under usual settings. The condition for exposure and development may vary.

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