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

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-SLC18A3/VAChT

Code No.	Quantity	Form
BMP048	50 µL	Affinity Purified

BACKGROUND: SLC18A3, also known as the vesicular acetylcholine transporter (VAChT), belongs to the family of vesicular monoamine transporters (VMATs). It is localized to the cell membranes in the peripheral and central cholinergic nervous systems and transports the neurotransmitter acetylcholine, which is synthesized by the enzyme choline acetyltransferase from the compounds choline and acetyl-CoA, into the synaptic secretory vesicles. The transport of acetylcholine occurs via a proton gradient established by vacuolar ATPase. In addition, the *SLC18A3* gene is located within the first intron of the choline acetyltransferase gene, and protein kinase A appears to regulate transcription at this cholinergic gene locus.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC18A3.

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 -20ºC.

REACTIVITY: This antibody can be used to stain endogenous antigen in paraffin embedded human tissues including kidney by Immunohistochemistry. The reactivity has been confirmed by intracellular Flow cytometry to detect the full length of human SLC18A3 transiently expressed in HEK 293T cells.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; 1:5,000
Heat treatment is necessary for staining paraffin embedded sections.
Autoclave; 125ºC 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; 1:200 (final concentration)

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

INTENDED USE:
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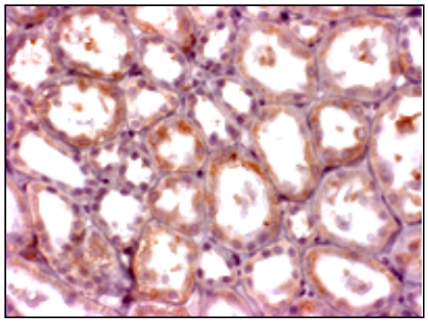
SPECIES CROSS REACTIVITY:

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

REFERENCES:

1) Elwary, S. M., *et al., J. Invest. Dermatol*. 126, 1879-1884 (2006)
2) Varoqui, H., and Erickson, J. D., J. Biol. Chem. **271**, 27229-27232 (1996)
3) Erickson, J. D., *et al., J. Biol. Chem*. **269**, 21929-21932 (1994)

kidney



Immunohistochemical detection of SLC18A3 on paraffin embedded section of human kidney with BMP048. *Multi pathological types tissue array (MBL) was used for this application.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

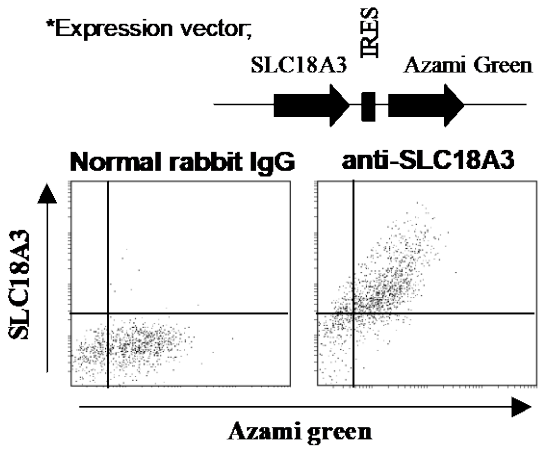
PROTOCOLS:

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 (PBS containing 0.5% BSA, 5% Normal goat serum) 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 60 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.



Flow cytometric analysis of intracellular SLC18A3 expression on 293T transiently expressing SLC18A3 and Azami green*. *The staining intensity of BMP048 is shown in the vertical axis with Azami Green fluorescence on the horizontal axis.*

(Positive controls for Immunohistochemistry; kidney)

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.