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

MBL

POLYCLONAL ANTIBODY

# Anti-SLC17A9 isoform 2 (VNUT) (Human) pAb

| Code No. | Quantity | Form |
|---|---|---|
| BMP079 | 100 µL | Affinity Purified |

**BACKGROUND:** Adenosine-5'-triphosphate (ATP), which is known to play an important role in intracellular energy transfer, also functions extracellularlly as a chemical transmitter in purinergic signal transmission in neurons and endocrine cells. ATP is stored in secretory vesicles and subsequently exocytosed to stimulate various purinergic responses including those of autonomic functions, pain and mechanosensory transduction, neural-glial interactions, as well as platelet aggregation. Solute carrier family 17 (SLC17) has been originally classified as the vesicular glutamate transporter family, but SLC17A9 isoform 2/VNUT may play a role in the vesicular storage of ATP and other nucleotides in secretory vesicles such as adrenal chromaffin granules and synaptic vesicles.

**SOURCE:** This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC17A9 isoform 2.

**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 the small intestine and kidney by Immunohistochemistry. The reactivity has been confirmed by intracellular Flow cytometry to detect the full length of human SLC17A9 isoform 2 transiently expressed in HEK 293T cells.

## APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; Not tested
Immunohistochemistry; 1:500
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; 1:500 (final concentration)

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

## INTENDED USE:

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

## REFERENCES:

1) Shin, Y. H.., *et al*., *Biochem Biophys Res Commun* **429**, 163-167 (2012) [IHC]
2) Chaudhury, A., *et al*., *Am J Physiol Gastrointest Liver Physiol* **302**, G598-607 (2012) [IHC]
3) Sawada, K., *et al., PNAS* **105**, 5683-5686 (2008)
4) Rudnick G., *et al., PNAS* **105**, 5949-5950 (2008)

## SPECIES CROSS REACTIVITY:

| Species | Human | Mouse* | Rat* |
|---|---|---|---|
| Tissues | small intestine, kidney | Not tested | Not tested |
| Reactivity on IHC | + | | |

*It is reported that this antibody can be used in rat [1] and mouse[2].

## RELATED PRODUCTS:

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## PROTOCOLS:

**Immunohistochemical staining for paraffin-embedded sections**

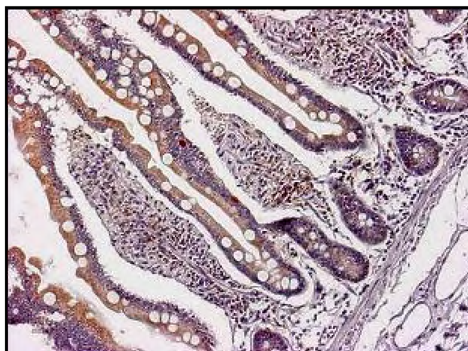
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Autoclave:
   Heat the slides immersed in retrieval solution [10 mM citrate 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% $H_2O_2$ in PBS 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.

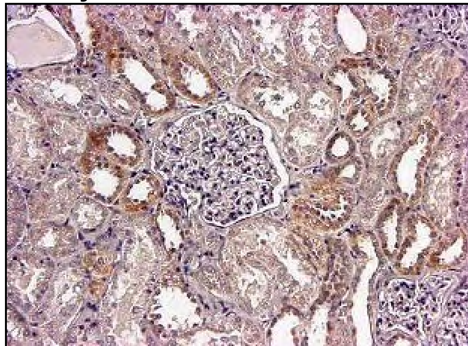
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
   **Note:** It is essential for every laboratory to determine the optional titers of the primary antibody to obtain the best result.
8) Incubate the sections overnight at 4°C.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Histostar (Ms+Rb) (MBL, code no. 8460). Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 10 minutes with DAB substrate solution (MBL, code no. 8469). *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 controls for Immunohistochemistry; small intestine, kidney)

small intestine



kidney

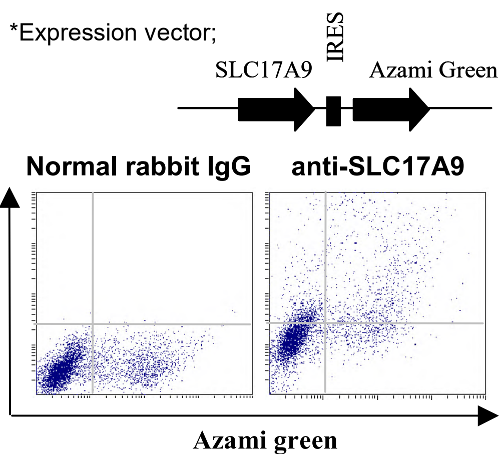


***Immunohistochemical detection of SLC17A9 on paraffin embedded section of human small intestine and kidney with BMP079.***

## 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 0.5% BSA.
2) Resuspend the cells with PBS containing 0.5% BSA ($5x10^6$ cells/mL).
3) Add 100 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 in PBS to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
5) Wash the cells twice with PBS containing 0.5% BSA.
6) Add 100 µL 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 (20~25°C).
7) Add 50 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 room temperature.
8) Add 50 µL of the primary antibody at a titer as suggested in the **APPLICATIONS** diluted with blocking buffer. Mix well and incubate for 2 hours at room temperature.
9) Wash the cells 3 times with PBS containing 0.1% Triton X-100, 0.5% BSA.
10) Add 50 µL of PE conjugated anti-rabbit IgG antibody diluted with blocking buffer. Mix well and incubate in the dark for 1 hour at room temperature.
11) Wash the cells 3 times with PBS containing 0.1% Triton X-100, 0.5% BSA.
12) Resuspend the cells with 500 µL of PBS containing 0.5% BSA, 2 mM EDTA analyze by a flow cytometer.




***Flow cytometric analysis of intracellular SLC17A9 expression on 293T transiently expressing SLC17A9 and Azami green*.*** *The staining intensity of BMP079 is shown in the vertical axis with Azami Green fluorescence on the horizontal axis.*