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

POLYCLONAL ANTIBODY

Anti-SLC40A1 (FPN1) (Human) pAb

Code No.	Quantity	Form
BMP033	50 µL	Affinity Purified

BACKGROUND: SLC40A1, also known as ferroportin 1, belongs to the iron-regulated transporter family and absorbs iron from the diet into the bloodstream through the small intestine. The absorbed iron binds to transferrin in the blood and is then carried to tissues and organs throughout the body. SLC40A1 also transports iron out of reticuloendothelial cells, which are phagocytic immune cells present in the liver, spleen, and bone marrow. Mutations in the *SLC40A1* gene cause hemochromatosis, which is characterized by impaired iron regulation and disorders associated with iron deficiency or overload.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC40A1.

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 kidney by Immunohistochemistry. The reactivity has been confirmed by Western blotting, Immunocytochemistry, and intracellular Flow cytometry to detect the full-length of human SLC40A1 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 [10 mM Tris-HCl, 1 mM EDTA, containing 0.05% Tween-20 (pH 9.0)].
Immunocytochemistry; 1:1,000
Flow cytometry; 1:1,000 (final concentration)

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

INTENDED USE:

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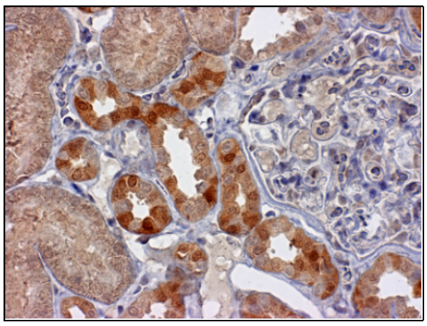
SPECIES CROSS REACTIVITY:

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

REFERENCES:

1) Zohn, I. E., *et al., Blood* **109**, 4174-4180 (2007)
2) Donovan, A., *et al., Cell Metab*. **1**, 191-200 (2005)
3) Domenico, I. D., *et al., PNAS.* **102**, 8955-8960 (2005)
4) Montosi, G., *et al., J. Clin. Invest.* **108**, 619-623 (2001)
5) Donovan, A., *et al., Nature* **403**, 776-781 (2000)
6) McKie, A. T., *et al., Mol. Cell* **5**, 299-309 (2000)

kidney



Immunohistochemical detection of SLC40A1 on paraffin embedded section of human kidney with BMP033. *Multi pathological types tissue array (MBL) was used for this application.*

PROTOCOLS:

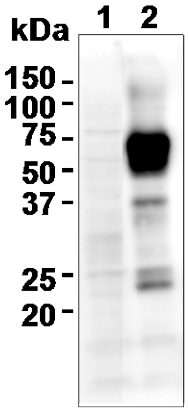
Immunohistochemical staining for paraffin-embedded sections

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 PBS containing 0.5%BSA and 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 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.

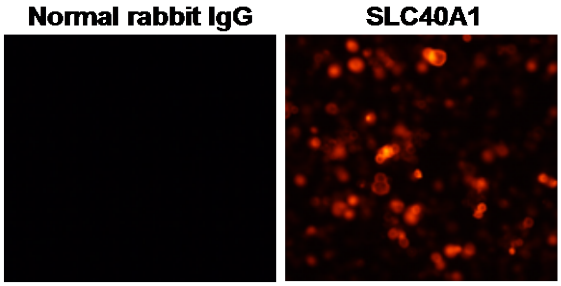
(Positive control for Immunohistochemistry; Human kidney)



Western blot analysis of SLC40A1 expression in Myc-tagged SLC40A1 transfected 293T (2) and parental cell (1) using BMP033.

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 the sample per lane in a 1 mm thick SDS-polyacrylamide gel for 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 manufacture'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 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 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.

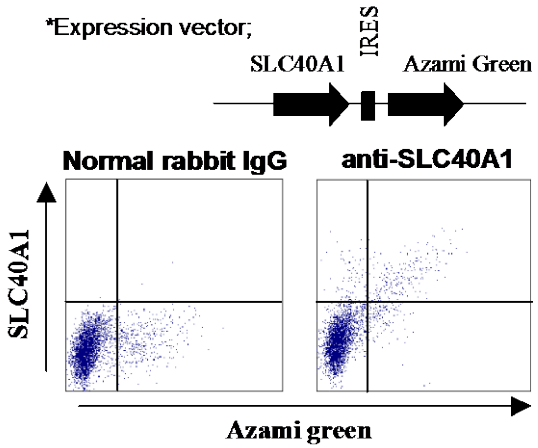


Immunocytochemical detection of SLC40A1 in 293T transiently expressing SLC40A1 with BMP033 (right) or normal rabbit IgG (left).

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 in PBS containing 4% paraformaldehyde (PFA) 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 SLC40A1 expression on 293T transiently expressing SLC40A1 and Azami green*. The staining intensity of BMP033 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.