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

POLYCLONAL ANTIBODY

Anti-SLC4A1/AE1

Code No.	Quantity	Form
BMP012	100 µL	Affinity Purified

BACKGROUND: SLC4A1/AE1/Band3/CD233, a member of the anion exchanger family, is the major glycoprotein of the erythrocyte membrane. SLC4A1 has an important role for the high rate exchange of chloride by bicarbonate ion during carbon dioxide transport by erythrocytes. Defective mutations of SLC4A1 gene have been reported to cause either the erythroid disorders including spherocytic hemolytic anaemia or ovalocytosis, or distal renal tubular acidosis. One SLC4A1 null human is also known with very severe anemia and nephrocalcinosis.

SOURCE: This antibody was affinity purified from rabbit serum. The rabbit was immunized with a synthetic peptide derived from human SLC4A1.

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 erythrocytes in paraffin embedded human tissues by immunohistochemistry. The reactivity of this antibody has been confirmed by Western blotting to detect the full-length of human SLC4A1 transiently expressed in HEK 293T cells.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
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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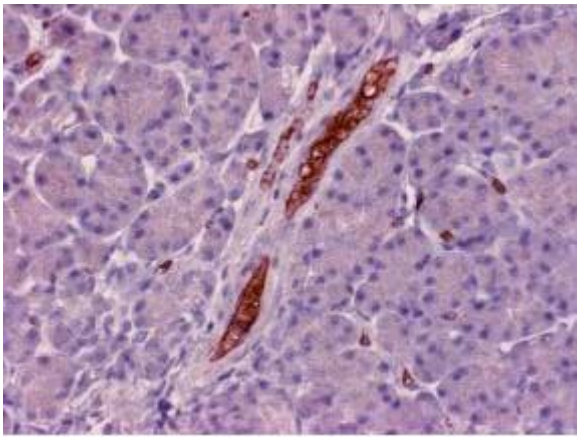
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	erythrocyte	Not Tested	Not Tested
Reactivity on IHC	+		

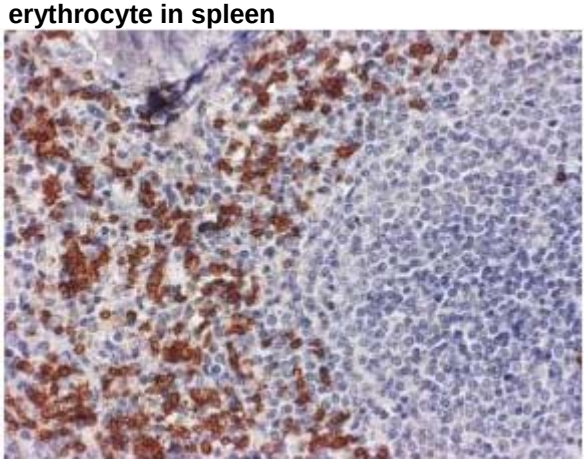
REFERENCES:

1) Shayakul, C., and Alper, S. L., *Clin. Exp. Nephrol.* **8**, 1-11 (2004)
2) Karet, F. E., *et al.*, *PNAS* **95**, 6337-6342 (1998)
3) Lux, S. E., *et al.*, *PNAS* **86**, 9089-9093 (1989)
4) Palumbo, A. P., *et al.*, *Am. J. Hum. Genet.* **39**, 307-316 (1986)

erythrocyte in pancreas



erythrocyte in spleen



Immunohistochemical detection of SLC4A1 on paraffin embedded section of erythrocyte in human pancreas and spleen with BMP012. *Multi pathological types tissue array (MBL) was used for this application.*

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

PROTOCOLS:

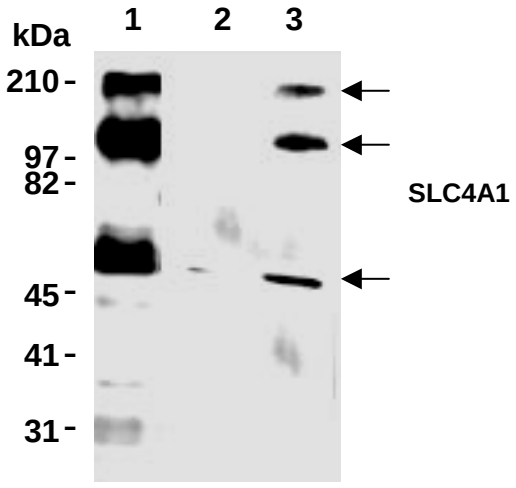
Immunohistochemical staining for paraffin-embedded sections

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
   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; erythrocyte)

SDS-PAGE & Western Blotting

1) Wash cells (approximately 2 x 10^6 cells) 3 times with PBS and resuspend 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 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 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 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 for 2 hours at room temperature with primary antibody diluted with PBS (pH 7.2) containing 2% skimmed milk 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 times).
9) Incubate the membrane with 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, and incubate the membrane 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 conditions for exposure and development may vary.



Western blot analysis of SLC4A1 expression in Myc-tagged SLC4A1 transfected 293T (1, 3) and parental cell (2) using anti-Myc-tag antibody (1, MBL; code no. M047-3) or BMP012 (2, 3).