Antibody Characterization Report for Equilibrative nucleoside transporter 1 (SLC29A1)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Equilibrative nucleoside transporter 1

Alternative protein name: SLC29A1

Gene name: *SLC29A1*

Uniprot: Q99808

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. This report guides researchers to select the most appropriate antibodies for SLC29A1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for SLC29A1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HAP1 was selected based on evidence of appropriate SLC29A1 expression [3]. HAP1 and HEK293 *SLC29A1* KO lines are available from Horizon Discovery and RESOLUTE, respectively, and were used in this study.

The authors do not provide an assessment of the quality of the tested antibodies as their respective performances are limited to our finite experimental conditions. The readers should interpret the present findings based on their own scientific expertise. The authors acknowledge that an antibody that demonstrates specificity in the stated test conditions can be suboptimal in a different experimental format or in cell lines that differ from those directly tested here.

Table 1: Summary of the SLC29A1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab182023**	GR3232722-2	AB_2885105	monoclonal	SP120	Rabbit	not provided	Other application
Abcam	ab223851**	GR3198810-3	AB_2885116	monoclonal	EPR20556	Rabbit	0.53	Wb
Proteintech	11337-1-AP	not provided	AB_2190784	polyclonal	-	Rabbit	0.16	Wb,IP
Bio-Techne	NBP2-41313	8125-1402	AB_2885156	polyclonal	-	Rabbit	1.00	Wb,IF

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence, **=recombinant antibody

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype	Comment
RESOLUTE	CE0002JumpIN WS	-	HEK293	WT	-
RESOLUTE	CE00C4-B	-	HEK293	*SLC29A1* KO[1]	See footnote
Horizon Discovery	C631	CVCL_Y019	HAP1	WT	-
Horizon Discovery	HZGHC000785c004	CVCL_TM74	HAP1	*SLC29A1* KO	-

[1] Please contact RESOLUTE (contact@re-solute.eu) to obtain this KO cell line

Figure 1: SLC29A1 antibody screening by immunoblot

A) Lysates of HEK293 (WT and *SLC29A1* KO) were prepared, and 100 µg of protein were processed for immunoblot with the indicated SLC29A1 antibodies. The Ponceau stained transfers of each blot are shown. All antibodies were diluted at 1/1000. Expected band size: ~50 kDa.

B) Lysates of HEK293 and HAP1 parental and *SLC29A1* KO were prepared, and 100 µg were processed for immunoblot with ab182023** at 1/1000 dilution. Longer exposure of the ~48 kDa area is shown in the cropped middle panel. **=recombinant antibody

Figure 2: SLC29A1 antibody screening by immunoprecipitation

A) HAP1 lysates were prepared, and immunoprecipitation was performed using 10 µl of ab182023** antibody or 2.0 µg of the other indicated SLC29A1 antibodies pre-coupled to Dynabeads protein G or protein A.

B) HAP1 lysates (WT and *SLC29A1* KO) were prepared, and immunoprecipitation was performed using 5 µl of ab182023** antibody pre-coupled to Dynabeads protein A. Samples were washed and processed for immunoblot with ab182023 at 1/500. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. **=recombinant antibody

Figure 3: SLC29A1 antibody screening by immunofluorescence

HAP1 WT and *SLC29A1* KO cells were labelled with a green or a far-red dye, respectively. Parental and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated SLC29A1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. Parental and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: ab182023** at 1/200; ab223851** at 1/500; 11337-1-AP at 1/200 ; NBP2-41313 at 1/200. Bars = 20 µm. **=recombinant antibody

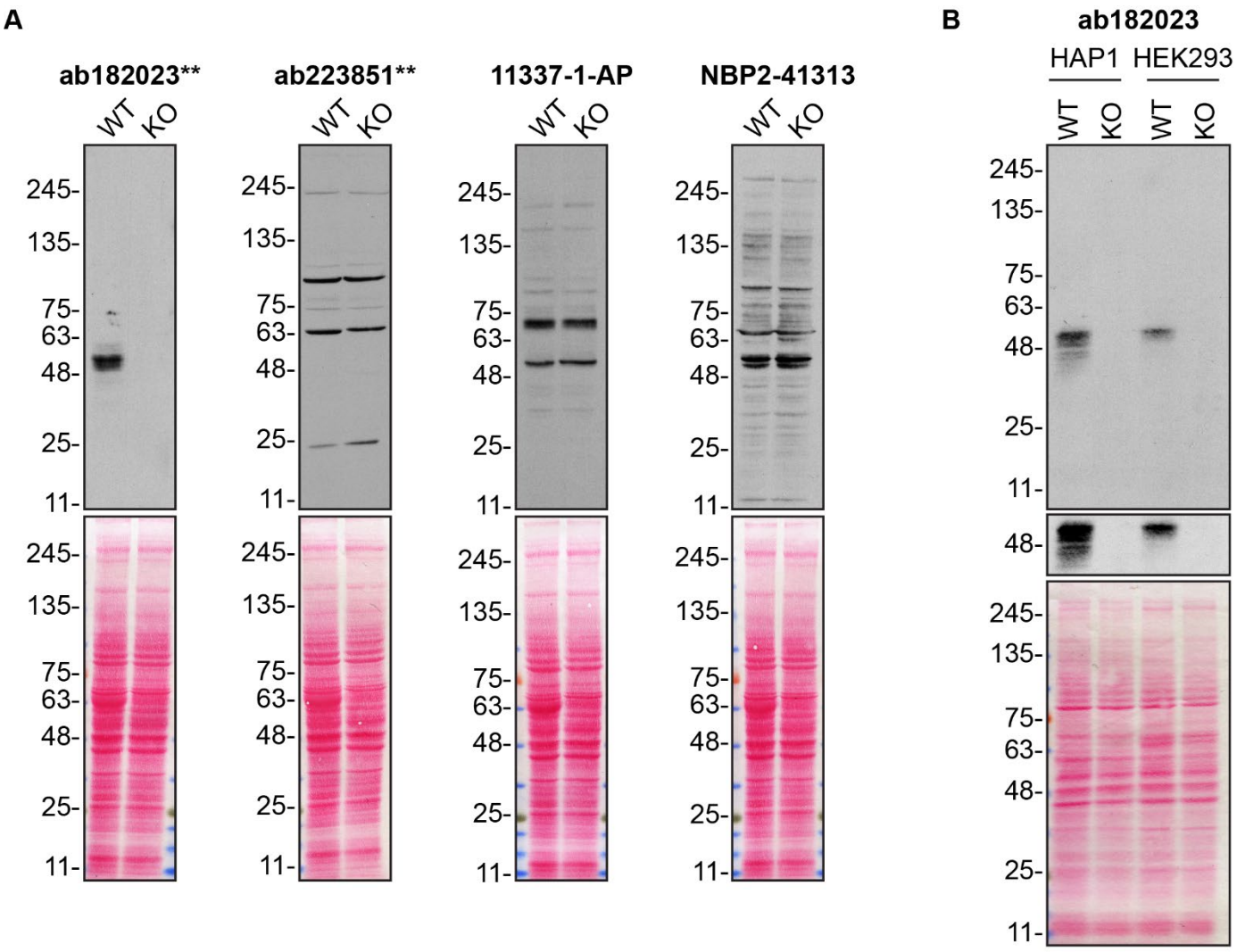

Figure 1: SLC29A1 antibody screening by immunoblot

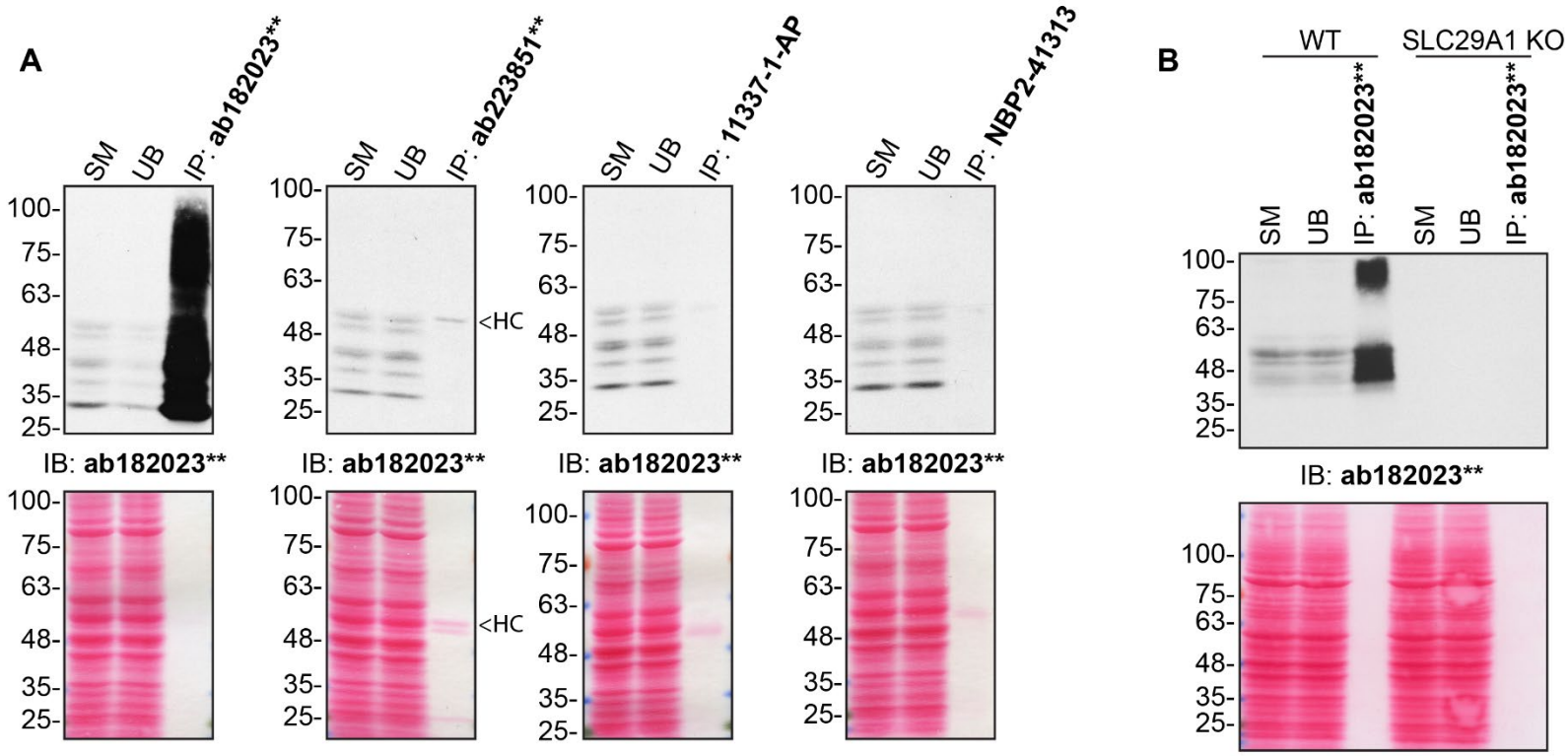

Figure 2: SLC29A1 antibody screening by immunoprecipitation

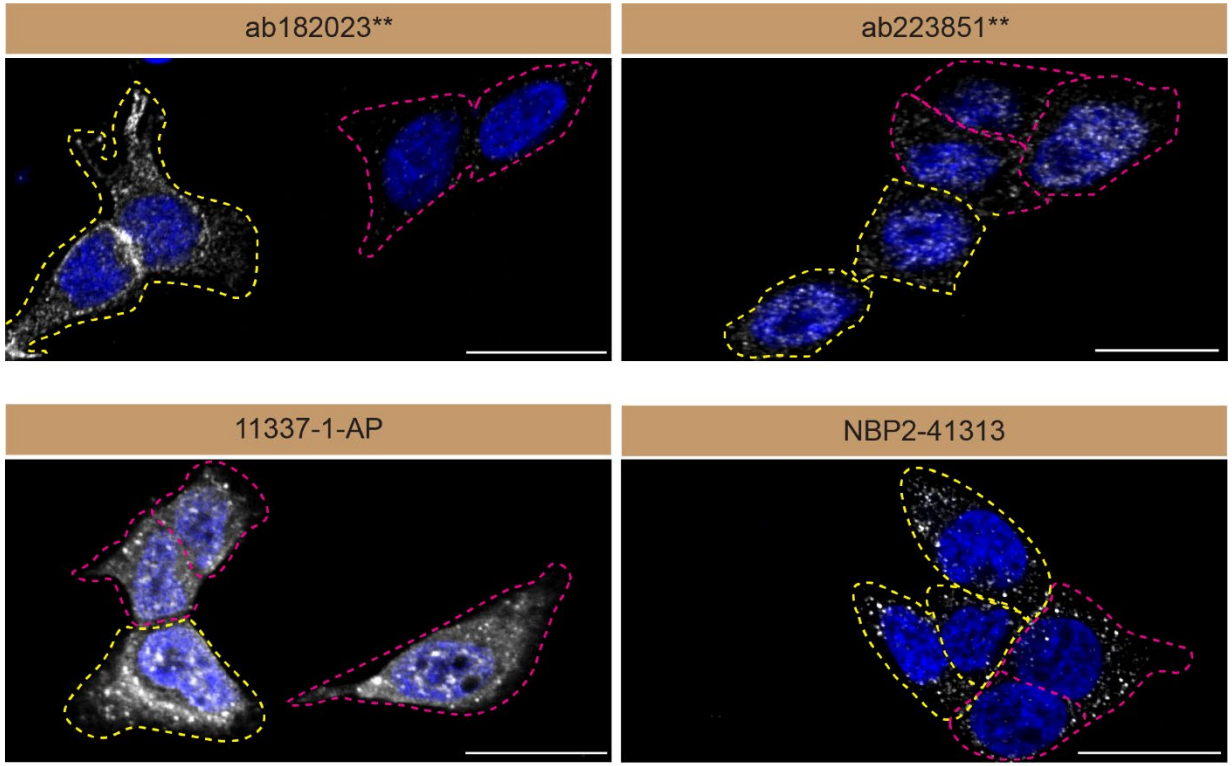

Figure 3: SLC29A1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All SLC29A1 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. Number 62-6520 and 65-6120). Alexa-555-conjugated goat anti-rabbit secondary antibody is from Thermo Fisher Scientific (cat. number A-21429)

Cell culture

HEK293 and HAP1 WT and *SLC29A1* KO were cultured in DMEM high glucose (GE Healthcare cat. number SH30081.01) containing 10% bovine calf serum (GE Healthcare cat. number SH30072.03), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Immunoblot

Immunoblots were performed as described in our standard operating procedure [4]. HEK293 and HAP1 (WT and *SLC29A1* KO) were collected in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor. Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at ~110,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Immunoblots were performed with large 4-15% gradient polyacrylamide gels and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau staining which is scanned to show together with individual immunoblot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% bovine serum albumin in TBS with 0,1% Tween 20 (TBST). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated at a dilution of ~0.2 µg/ml in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes are incubated with ECL from Pierce (cat. number 32106) prior to detection with HyBlot CL autoradiography films from Denville (cat. number 1159T41).

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure [5]. Antibody-bead conjugates were prepared by adding 2 µg or 5 µl - 10 µl of antibody at an unknown concentration to 500 ul of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with with 30µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Pierce IP Lysis Buffer was supplemented with the Halt Protease Inhibitor Cocktail 100X from Thermo Fisher Scientific (cat. number 78446) at a final concentration of 1x. Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

HAP1 WT were collected in Pierce IP buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) supplemented with protease inhibitor. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1.0 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. Following centrifugation, the unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP lysis buffer and processed for SDS-PAGE and immunoblot on 8-16% or 4-15% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a dilution of 0.4 µg/ml for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot.

Immunofluorescence

Immunofluorescence was performed mostly as described in our standard operating procedure [6]. HAP1 WT and *SLC29A1* KO were labelled with a green dye (Abcam cat. number ab176735) or a deep red dye (Abcam cat. ab176736), respectively. WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator. Cells were fixed with 4% PFA (in PBS) for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0,1% Triton X-100 for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Coverslips were incubated face down on a 50 µl drop (on paraffin film in a moist chamber) with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary SLC29A1 antibodies O/N at 4°C. Cells were washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature. Cells were washed 3 × 10 min with IF buffer and once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Zeiss LSM 880 laser scanning confocal microscope equipped with a Plan-Apo 40x oil objective (NA = 1.40). All cell images represent a single focal plane. Figures were prepared using Adobe Photoshop to adjust contrast, apply 1 pixel Gaussian blur and then assembled with Adobe Illustrator.

References

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