

Antibody Characterization Report for Solute carrier family 2, facilitated glucose transporter member 6 (GLUT-6)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Solute carrier family 2, facilitated glucose transporter member 6

Alternative protein name: Glucose transporter type 6

Alternative protein name (short): GLUT-6

Gene name: *SLC2A6*

Uniprot: Q9UGQ3

The goal of this report is to guide researchers to select the most appropriate antibodies for Solute carrier family 2, facilitated glucose transporter member 6 (GLUT-6). We used an antibody characterization pipeline¹ based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for G6PT by immunoblot (Western blot). HAP1 and HuH-7 *SLC2A6* KO available at Horizon Discovery and RESOLUTE, respectively, were used for antibody screening.

- 1 Laflamme, C. *et al.* Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72. *Elife* **8**, doi:10.7554/eLife.48363 (2019).

Table 1: Summary of the GLUT-6 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration ($\mu\text{g}/\mu\text{l}$)
Thermo	MA5-24979	VJ3101881	AB_2723654	monoclonal	OTI7E3	mouse	1.47
Abcam	ab119272	GR3250342-1	AB_10903618	polyclonal	-	rabbit	0.60

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype	Comment
RESOLUTE	CE00EH-8	-	HCT116	WT	-
RESOLUTE	CE04S4-2	-	HCT116	<i>SLC2A6</i> KO ^a	See footnote
HAP1	C631	CVCL_Y019	HAP1	WT	-
HAP1	HZGHC005485c005	CVCL_XT14	HAP1	<i>SLC2A6</i> KO	-

^a Please contact RESOLUTE (contact@re-solute.eu) to obtain this KO cell line.

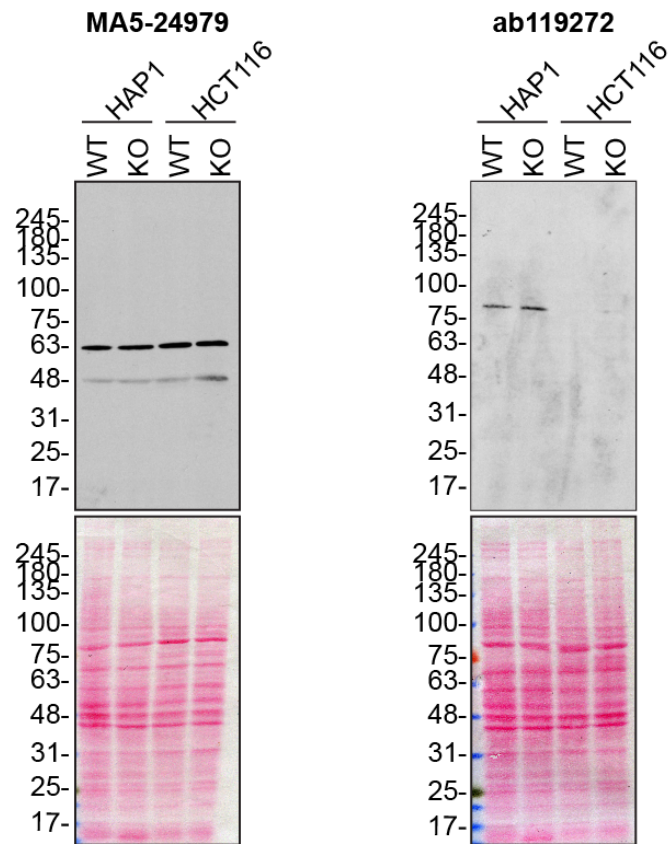


Figure 1: GLUT-6 antibody screening by immunoblot

Figure 1: Analysis of GLUT-6 antibodies by immunoblot.

Lysates of HCT116 and HAP1 (WT or *SLC2A6* KO) were prepared and 100 µg of protein were processed for immunoblot with the indicated GLUT-6 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used of tested antibodies was 1/1000. Expected band size: ~55 kDa.

Materials and methods

Antibodies

All GLUT-6 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520).

Cell culture

HAP1 and HCT116 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

HAP1 and HCT116 WT and *SLC2A6* KO cells were collected in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM EDTA, 1% Triton X-100, 0.5% 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).