Antibody Characterization Report for Glucose-6-phosphate exchanger SLC37A4 (G6PT)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Glucose-6-phosphate exchanger SLC37A4

Protein name (short): G6PT

Alternative protein names: Glucose-5-phosphate transporter, Glucose-6-phosphate translocase, Solute carrier family 37 member 4, Transformation-related gene 19 protein, TRG-19

Gene name: *SLC37A4*

Uniprot: O43826

The goal of this report is to guide researchers to select the most appropriate antibodies for Glucose-6-phosphate exchanger SLC37A4 (G6PT). We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for G6PT by immunoblot (Western blot). HuH-7 *SLC37A4* KO available at RESOLUTE was 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 G6PT antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)
Biorbyt	orb6969	FT15483	AB_10929163	polyclonal	-	Rabbit	1.00
Biorbyt	orb578942	AS7674	AB_2885137	polyclonal	-	Rabbit	not provided
Biorbyt	orb419876	SBA5457	AB_2885135	polyclonal	-	Rabbit	0.40
Biorbyt	orb631272	A4002	AB_2885138	polyclonal	-	Rabbit	not provided
Proteintech	20612-1-AP	not provided	AB_10858386	polyclonal	-	Rabbit	0.18
Bio-Techne	NBP2-93652	1158640301	AB_2885162	polyclonal	-	Rabbit	1.60
Bio-Techne	NBP1-59877	QC14202-160421	AB_11003770	polyclonal	-	Rabbit	0.50
Thermo	PA5-58599	VJ3109409B	AB_2647489	polyclonal	-	Rabbit	0.20

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype	Comment
RESOLUTE	CE0003HUH-7 WS	-	HuH-7	WT	-
RESOLUTE	CE006-Q	-	HuH-7	*SLC37A4* KO[a]	See footnote

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

Figure 1: Analysis of G6PT antibodies by immunoblot.

Lysates of HuH-7 WT and *SLC37A4* KO were prepared and 200 µg (orb6969, orb578942, orb419876, orb631272) or 100 µg (20612-1-AP, NBP2-93652, NBP1-59877, PA5-58599) of protein were processed for immunoblot with the indicated G6PT antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used was 1/1000 for each tested antibody. Expected band size: ~46 kDa.

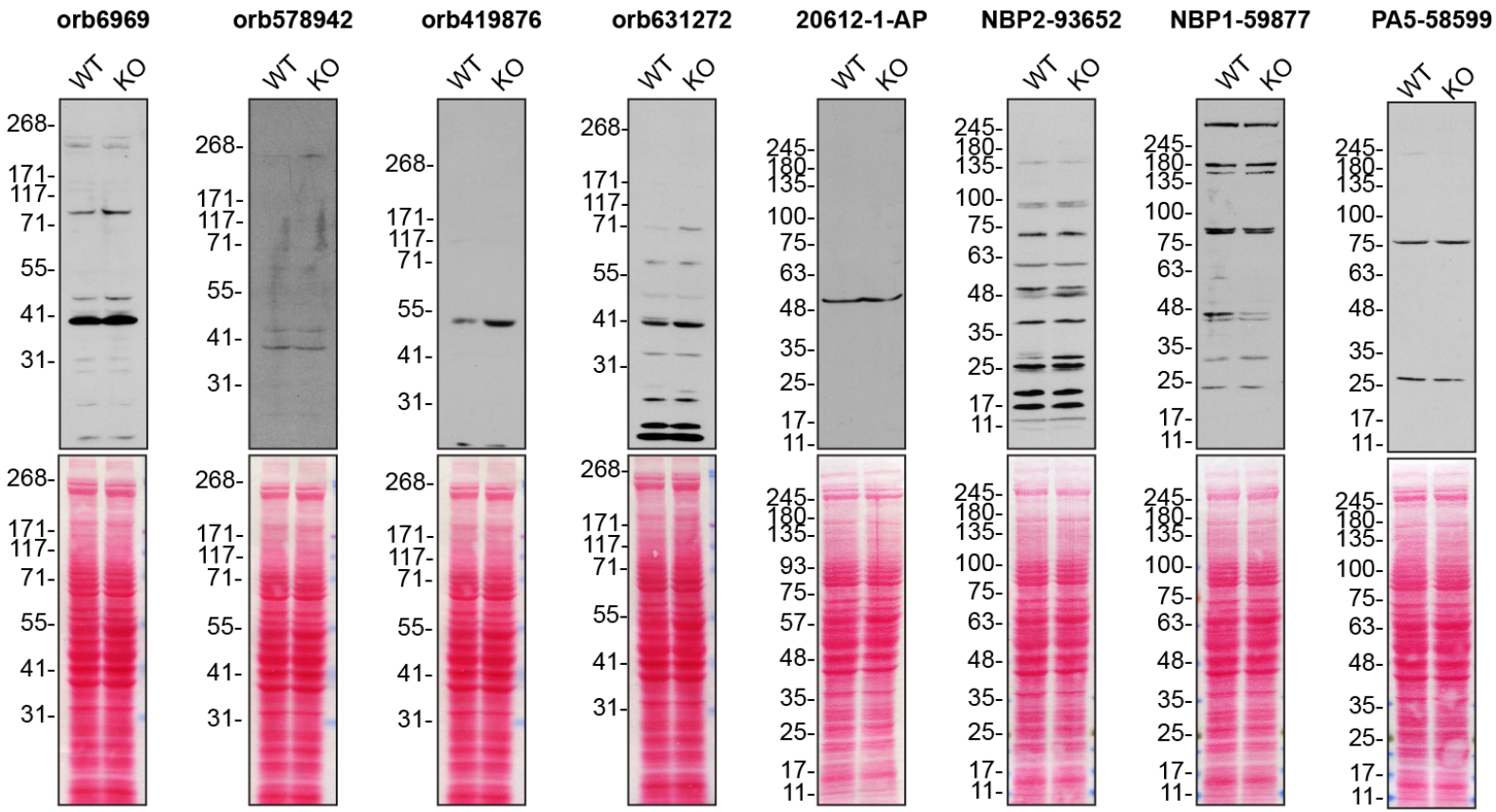

Figure 1: G6PT antibody screening by immunoblot

Materials and methods

Antibodies

All G6PT 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 lines

HuH-7 WT and KO lines 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

HuH-7 WT and *SLC37A4* KO 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.

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).