Antibody Characterization Report for Very long-chain acyl-CoA synthetase (VLCS)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Very long-chain acyl-CoA synthetase

Recommended protein name (short): VLCS

Alternative protein names: VLACS, Arachidonate--CoA ligase, Fatty acid transport protein 2, FATP-2, “Fatty-acid-coenzyme A ligase, very long-chain 1”, Long-chain-fatty-acid--CoA ligase, Phytanate--CoA ligase, Solute carrier family 27 member 2, THCA-CoA ligase, Very long-chain-fatty-acid-CoA ligase

Gene name: *SLC27A2*

Uniprot: O14975

The goal of this report is to guide researchers to select the most appropriate antibodies for Very long-chain acyl-CoA synthetase (VLCS). We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for VLCS by immunoblot (Western blot). HEK293 *SLC27A2* 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 VLCS antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)
Proteintech	14048-1-AP	not provided	AB_2239416	polyclonal	-	Rabbit	0.21
Thermo	MA5-31907	VJ3101187	AB_2787530	monoclonal	6B3A2	Mouse	1.00
Bio-Techne	MAB4659	CAMU219051	AB_2302055	monoclonal	466106	Mouse	0.50
Abcam	ab228784	GR3206698-22	AB_2885117	polyclonal	-	rabbit	1.00
Abcam	ab175373	GR220764-14	AB_2716562	monoclonal	6B3A9	mouse	1.00

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype	Comment
RESOLUTE	CE0002JumpIN WS	-	HEK293	WT	-
RESOLUTE	CE006C-C	-	HEK293	*SLC27A2* KO[a]	See footnote

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

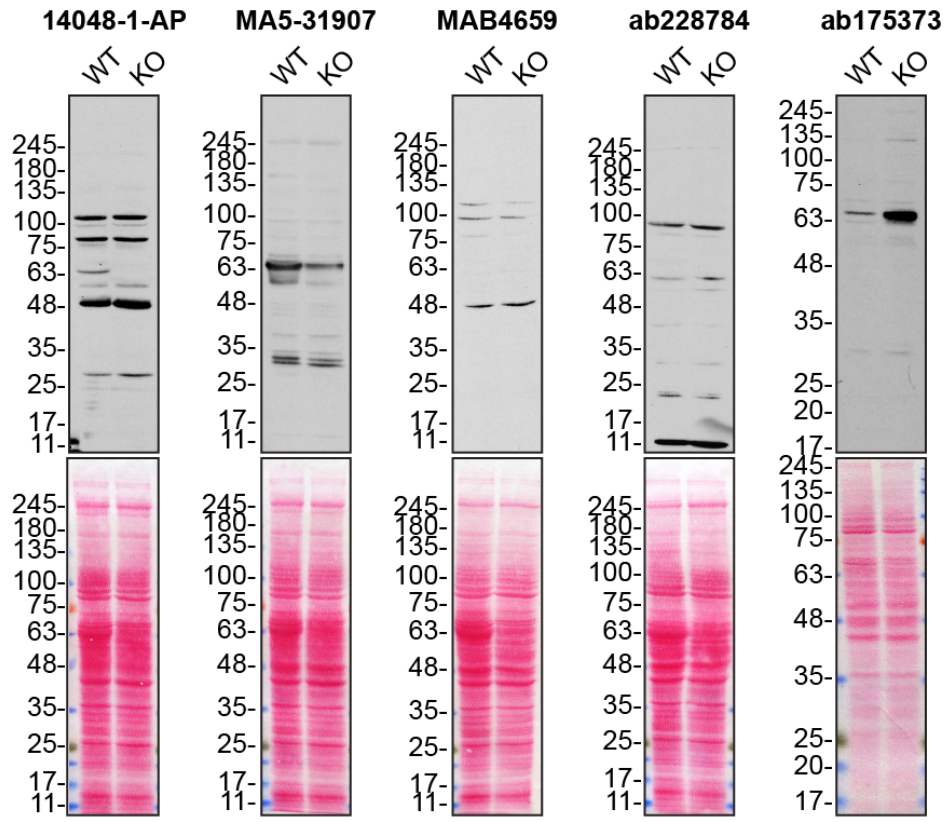

Figure 1: VLCS antibody screening by immunoblot

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Lysates of HEK293 WT and *SLC27A2* KO were prepared and 100 µg of protein were processed for immunoblot with the indicated VLCS antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used was 1/1000 for each tested antibody. Expected band size: ~70kDa.

Materials and methods

Antibodies

All VLCS 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

HEK293 WT and *SLC27A2* 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

HEK293 WT and *SLC27A2* 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).