

Antibody Characterization Report for Reduced folate transporter (FOLT)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Reduced folate transporter

Recommended protein name (short): FOLT

Alternative protein names: Intestinal folate carrier 1, IFC-1, Placental folate transporter, Reduced folate carrier protein, RFC, hRFC, Reduced folate transporter 1, RFT-1, Solute carrier family 19 member 1

Gene name: *SLC19A1*

Uniprot: P41440

The goal of this report is to guide researchers to select the most appropriate antibodies for Reduced folate transporter (FOLT). We used an antibody characterization pipeline¹ based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for FOLT by immunoblot (Western blot). We used already prepared cell lysates of WT and FOLT KO cell lines from available at Abcam.

- 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 FOLT antibodies tested

Company	Catalog number	Lot number	RRID	Clonality	Clone ID	Host	Concentration (µg/µl)
Bio-Techne	MAB8450	CIVJ0120011	AB_2885152	monoclonal	890513	mouse	0.50
Abcam	ab272354	GR3339784-4	AB_2885134	polyclonal	-	rabbit	0.50

Table 2: Summary of the SLC19A1 cell lysates used

Institution	Catalog number	Cell line	genotype
Abcam	ab255553	HEK293T	WT
Abcam	ab260719	HEK293T	SLC19A1 KO

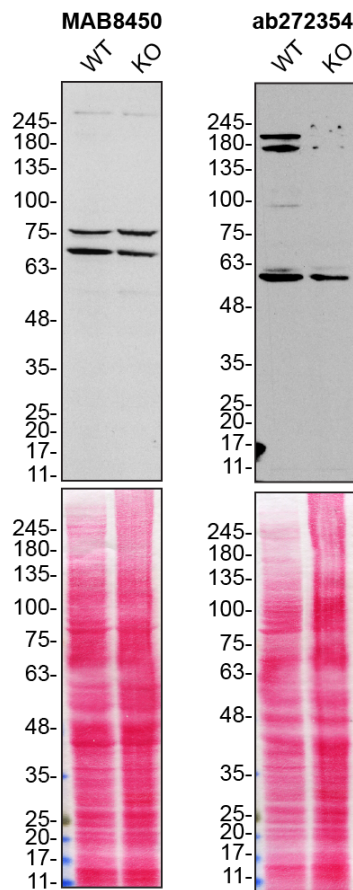


Figure 1: FOLT antibody screening by immunoblot

Figure 1: Analysis of FOLT antibodies by immunoblot.

Lysates of HCT116 and HAP1 (WT or KO) were prepared and 100 μ g of protein were processed for immunoblot with the indicated FOLT antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used was 1/1000. Expected band size: ~65 kDa.

Materials and methods

Antibodies

The FOLT 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).

Immunoblot

HEK293 WT and *SLC19A1* KO lyophilised lysates were reconstituted in water. 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).