

Antibody Characterization Report for Endothelin-converting enzyme 1

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Endothelin-converting enzyme 1

Gene name: *ECE1*

Uniprot: P42892

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 Endothelin-converting enzyme 1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Endothelin-converting enzyme 1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. A U-87MG *ECE1* KO line from Abcam was used in this study. Expression of Endothelin-converting enzyme 1 protein in U-87MG is adequate as determined by searching DepMap [3, 4].

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 Endothelin-converting enzyme 1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab71829	GR3458164-1	AB_2277809	polyclonal	-	rabbit	0.9	Wb
ABclonal	A5638	209770301	AB_2766398	polyclonal	-	rabbit	2.27	Wb,IF
Aviva Systems Biology	ARP76105	QC48429-42025	AB_2925163	polyclonal	-	rabbit	0.5	Wb
Bio-Techne	MAB1784*	WQO0208121	AB_2097861	monoclonal	303908	rat	0.5	Wb
Proteintech	26088-1-AP	26392	AB_2880371	polyclonal	-	rabbit	0.2	Wb
Thermo Fisher Scientific	PA5-109672	WJ3417791B	AB_2855083	polyclonal	-	rabbit	2.27	Wb,IF

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

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (CellSaurus)	Cell line	Genotype
ATCC	HTB-14	CVCL_0022	U-87MG	WT
Abcam	-	CVCL_C6JA	U-87MG	<i>ECE1</i> KO

Figure 1: Endothelin-converting enzyme 1 antibody screening by immunoblot.

Lysates of U-87MG WT and *ECE1* KO were prepared, and 20 µg of protein were processed for immunoblot with the indicated Endothelin-converting enzyme 1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab71829 at 1/500, A5638 at 1/1000, ARP76105 at 1/500, MAB1784* at 1/1000, 26088-1-AP at 1/1000, and PA5-109672 at 1/1000. Predicted band size: 87 kDa. *=monoclonal antibody

Figure 2: Endothelin-converting enzyme 1 antibody screening by immunoprecipitation.

U-87MG lysates were prepared, and immunoprecipitation was performed using 2.0 µg of the indicated Endothelin-converting enzyme 1 antibodies pre-coupled to Dynabeads protein G or protein A. Samples were washed and processed for immunoblot with the indicated Endothelin-converting enzyme 1 antibody. For immunoblot, A5638 was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody

Figure 3: Endothelin-converting enzyme 1 antibody screening by immunofluorescence.

U-87MG WT and *ECE1* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio in a 96-well plate with optically clear flat-bottom. Cells were stained with the indicated Endothelin-converting enzyme 1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. 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 blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Antibody dilution used: ab71829 at 1/1000, A5638 at 1/2000, ARP76105 at 1/500, MAB1784* at 1/500, 26088-1-AP at 1/200, and PA5-109672 at 1/2000. Bars = 10 µm. *=monoclonal antibody

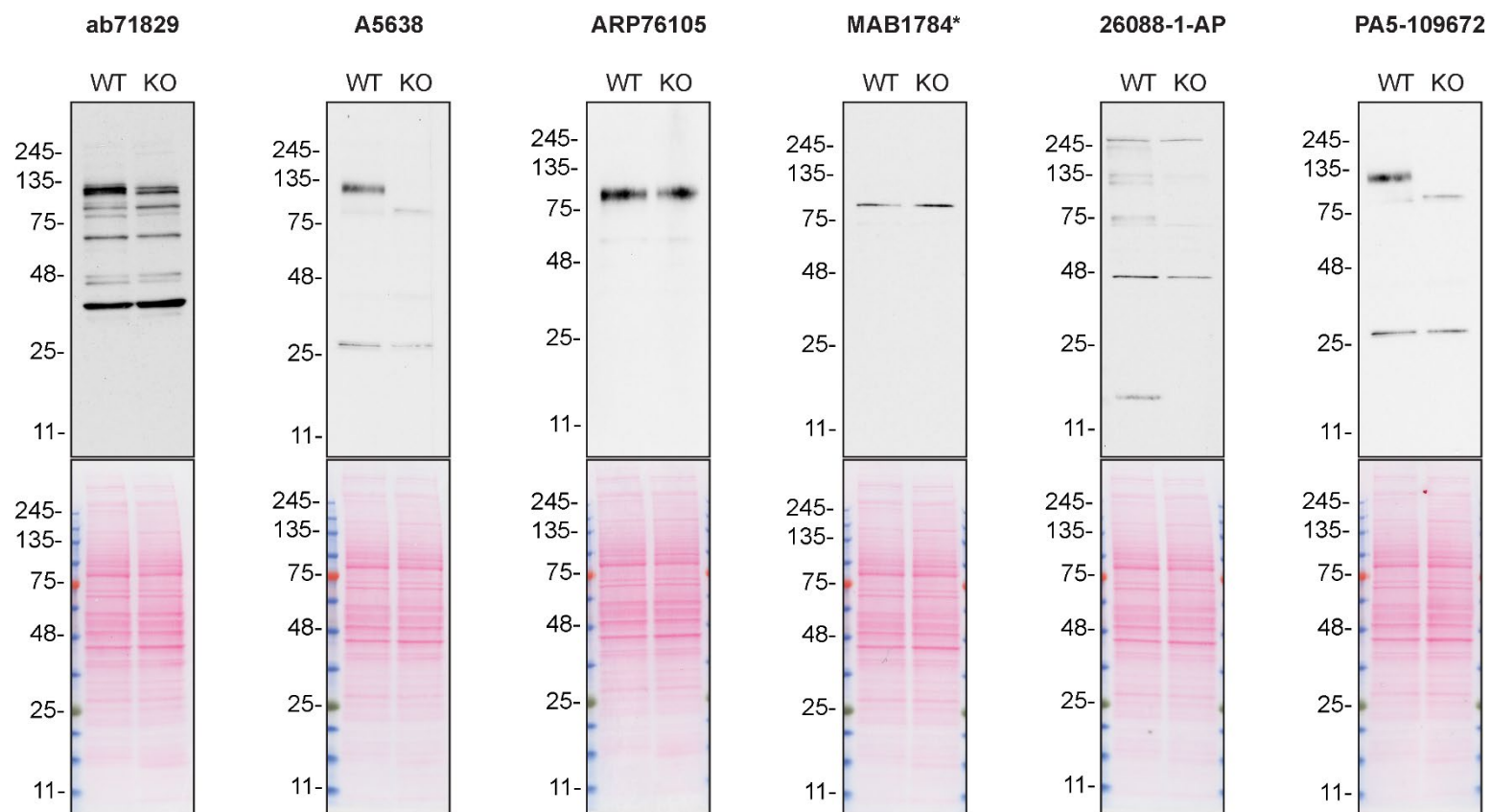


Figure 1: Endothelin-converting enzyme 1 antibody screening by immunoblot

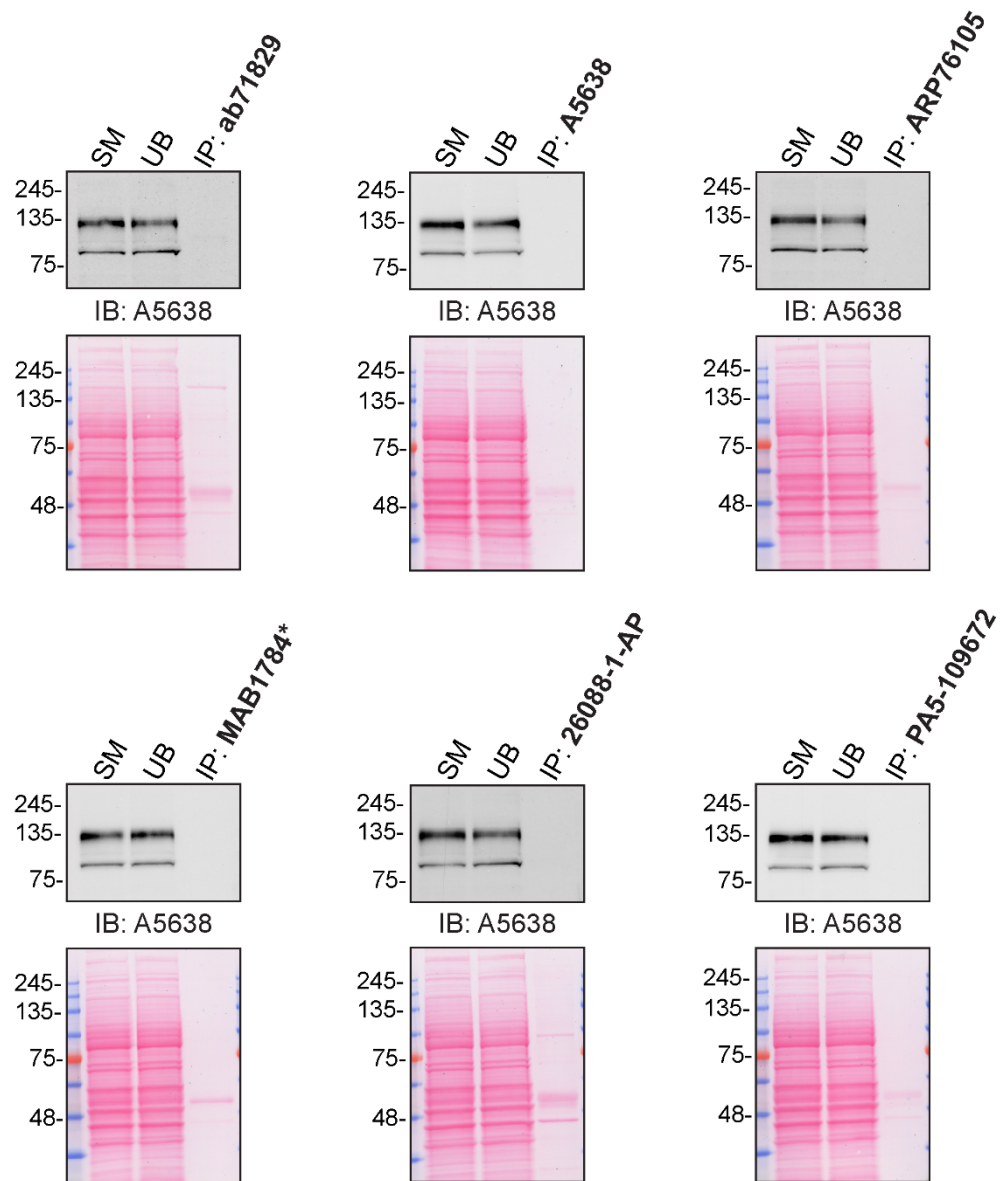


Figure 2: Endothelin-converting enzyme 1 antibody screening by immunoprecipitation

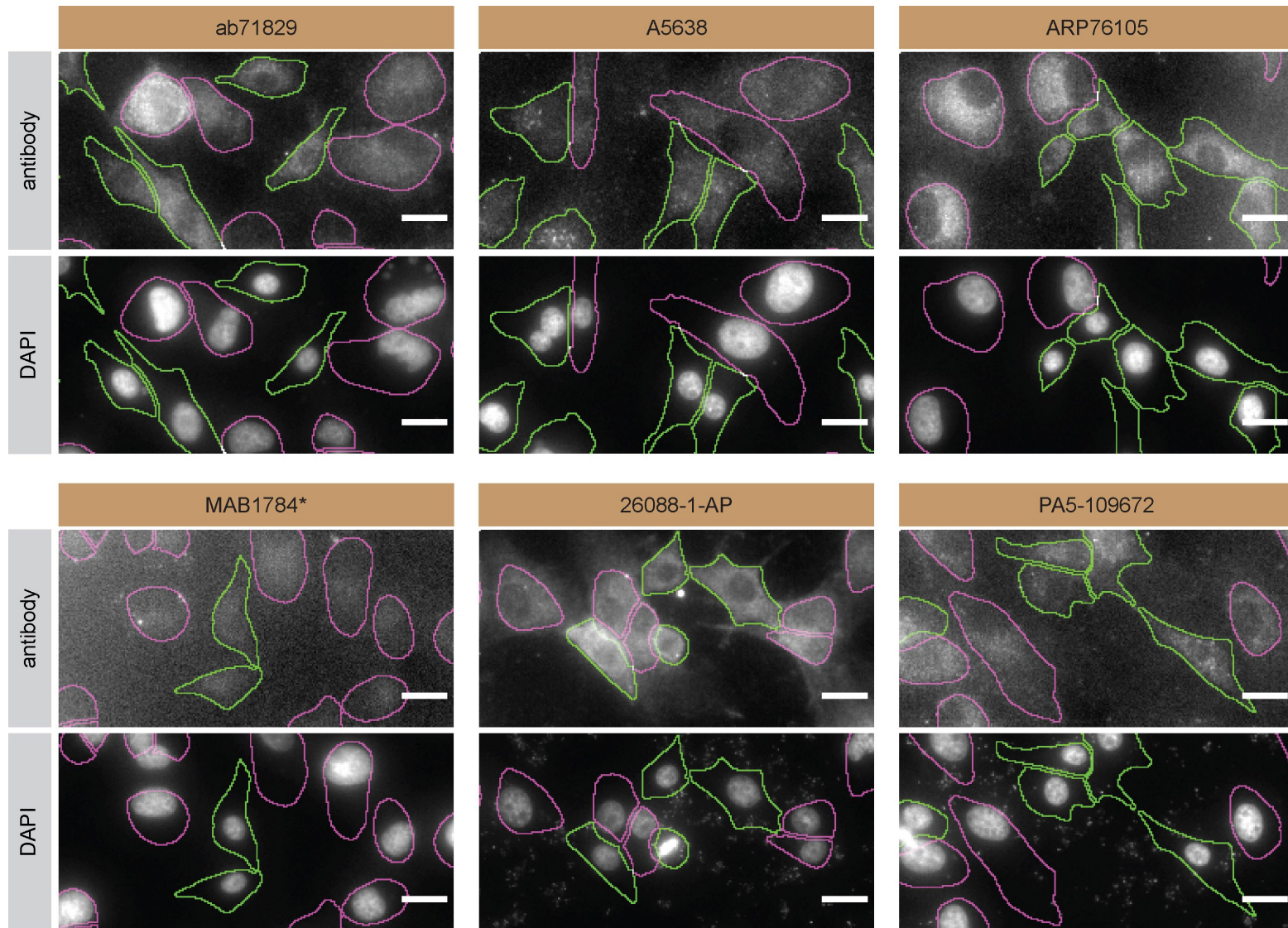


Figure 3: Endothelin-converting enzyme 1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All tested Endothelin-converting enzyme 1 antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse, anti-rabbit and anti-rat antibodies are from Thermo Fisher Scientific (cat. number 62-6520, 65-6120 and 31470, respectively). Alexa-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. U-87MG *ECE1* KO clone was generated using two guide RNAs to introduce 131 bp deletion in Exon 4 of the *ECE1* gene (sequence guide 1: CTGAGACACAAGCTTCGCTC, sequence guide 2: CCCTGATGGCCACTCACGCT).

Cell culture

Cells were cultured in DMEM high glucose (GE Healthcare cat. number SH30081.01) containing 10% fetal bovine serum (Wisent, cat. number 080450), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Antibody screening by immunoblot

Immunoblots were performed as described in our standard operating procedure [5]. U-87MG (WT and *ECE1* 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 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number 78429). 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 precast midi 4-20% Tris-Glycine polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX) ran with Tris/Glycine/SDS buffer from bio-Rad (cat. number 1610772), loaded in Laemmli loading sample buffer from Thermo Fisher Scientific (cat. number AAJ61337AD) 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) from Cell

Signaling (cat. number 9997). 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 Pierce ECL from Thermo Fisher Scientific (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 [6]. Antibody-bead conjugates were prepared by adding 2 µg to 500 µl of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30 µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) from Thermo Fisher Scientific (cat. number 10002D and 10004D, respectively). Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

U-87MG 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. 0.5 ml aliquots at 2.0 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. 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 precast midi 4-20% Tris-Glycine polyacrylamide gels from Thermo Fisher Scientific (cat. number WXP42012BOX). Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a concentration of 0.3 µg/ml.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure [7]. U-87MG WT and *ECE1* KO were labelled with a green and a far-red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific (cat. number C2925 and C34565). WT and KO cells were plated in 96-well plate with optically clear flat-bottom (Perkin Elmer, cat. number 6055300) as a mosaic and incubated for 24 hrs in a cell culture incubator. Cells were fixed in 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. Cells were incubated with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Endothelin-converting enzyme 1 antibodies O/N at 4°C. Cells were then 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 with DAPI. Cells were washed 3 × 10 min with IF buffer and once with PBS.

Images were acquired on an ImageXpress micro widefield high-content microscopy system (Molecular Devices), using a 20x NA 0.45 air immersion objective and scientific CMOS camera, equipped with 395, 475, 555 and 635 nm solid state LED lights (Lumencor Aura III light engine) and bandpass filters to excite DAPI, Cellmask Green, Alexa568 and Cellmask Red, respectively. Images had pixel sizes of 0.68 x 0.68 microns. Three images per field were acquired at a z-interval of 4 microns. Then, best focus intensity projections were generated from the z-stack. Segmentation was carried out separately on maximum intensity projections of Cellmask channels using CellPose 1.0, and masks were used to generate outlines and for intensity quantification. Figures were assembled with Adobe Illustrator.

References

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