Antibody Characterization Report for Angiotensin-converting enzyme (ACE)

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Angiotensin-converting enzyme

Alternative protein name: Dipeptidyl carboxypeptidase I, Kininase II

Gene name: *ACE*

Uniprot: P12821

We are a third-party organization with the mission to characterize commercial antibodies for all human protein through open science [1]. In this study, we characterized 15 Angiotensin-converting enzyme commercial antibodies for Western Blot and immunoprecipitation, using a standardized experimental protocol [2] based on comparing read-outs in knockout cell lines and isogenic parental controls. We identified many well-performing antibodies and encourage readers to use this report as a guide to select the most appropriate antibody for their specific needs. A custom-made SK-N-FI *ACE* KO line from Abcam was used in this study. SK-N-FI was selected based on evidence of appropriate Angiotensin-converting enzyme protein expression determined through DepMap [3, 4]. Western Blot results (Figure 1) show a decrease but not a complete loss of the Angiotensin-converting enzyme protein in the SK-N-FI *ACE* KO line.

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 cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype
ATCC	CRL-2142	CVCL_1702	SK-N-FI	WT
Abcam	-	CVCL_C7SN	SK-N-FI	*ACE* KO*
Horizon Discovery	C631	CVCL_Y019	HAP1	WT

*WB data (Figure 1) show a partial decrease in Angiotensin-converting enzyme protein in the *ACE* KO line, not a complete loss of the protein as would be expected for a KO line.

Table 2: Summary of the Angiotensin-converting enzyme antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Abcam	ab28311	GR319123-38	AB_726126	polyclonal	-	rabbit	1.0	Wb
Abcam	ab75762**	GR3436276-1	AB_1309952	recombinant-mono	EPR2757	rabbit	0.47	Wb
Abcam	ab254222**	GR3429423-1	n/a	recombinant-mono	EPR22291-247	rabbit	0.53	Wb
Abcam	ab270712*	GR3437856-1	n/a	monoclonal	ACE/3765	mouse	0.2	Wb
Aviva Systems Biology	ARP60161	QC31030-42424	n/a	polyclonal	-	rabbit	0.5	WB
Aviva Systems Biology	ARP79754	QC55240-42578	n/a	polyclonal	-	rabbit	0.5	Wb
Bio-Techne	MAB9291*	HYM0217041	AB_2257925	monoclonal	171409	mouse	0.5	Wb
Bio-Techne	NBP2-67111**	HN1221	n/a	recombinant-mono	JM59-32	rabbit	1.0	Wb
Bio-Techne	NBP3-08557	1636-2PABX210812	n/a	monoclonal	ACE/3762	mouse	1.0	Wb
GeneTex	GTX100923	39932	AB_1949562	polyclonal	-	rabbit	0.58	Wb
GeneTex	GTX130534	42046	AB_2886299	polyclonal	-	rabbit	0.25	Wb
Proteintech	24743-1-AP	60692	AB_2879701	polyclonal	-	rabbit	0.36	Wb,IP
Thermo Fisher Scientific	MA5-32741**	WG331663	AB_2810018	recombinant-mono	JM59-32	rabbit	1.0	Wb
Thermo Fisher Scientific	MA5-35099**	WI3377712	AB_2849004	recombinant-mono	ARC0577	rabbit	0.18	Wb
Thermo Fisher Scientific	PA5-86568	WI3378523A	AB_2803338	polyclonal	-	rabbit	1.0	Wb

Wb=Western Blot, IP= immunoprecipitation, IF=immunofluorescence, *=monoclonal antibody, **=recombinant antibody

Materials and methods

Antibodies

All the Angiotensin-converting enzyme antibodies tested are listed in Table 2. Peroxidase-conjugated goat anti-rabbit and anti-mouse are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 1. SK-N-FI *ACE* KO clone was generated at Abcam. Two guide RNAs were used to knockout the *ACE* gene (sequence guide 1: AGGAGCTGTATGAACCGATC, sequence guide 2: CTCCTAGTGCCCCATCGTGG).

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). Cells were starved in DMEM high-glucose containing L-glutamate and penicillin/ streptomycin.

Antibody screening by Western Blot on culture media

SK-N-FI WT and *ACE* KO (listed in Table 1) were washed 3x with PBS 1x and starved for ~18 hrs. Culture media were collected and centrifuged for 10 min at 500 x g to eliminate cells and larger contaminants, then for 10 min at 4500 x g to eliminate smaller contaminants. Culture media were concentrated by centrifuging at 4000 x g for 30min using Amicon Ultra-15 Centrifugal Filter Units with a membrane NMWL of 50kDa (MilliporeSigma cat. number UFC905024). Culture media were supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number 78429).

Western Blots were performed as described in our standard operating procedure [5]. Western Blots 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. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used. Proteins on the blots were visualized with Ponceau S staining (Thermo Fisher Scientific, cat. number BP103-10) which is scanned to show together with individual Western Blot. Blots were blocked

with 5% milk for 1 hr, and antibodies were incubated O/N at 4°C with 5% milk 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 were incubated with Pierce ECL from Thermo Fisher Scientific (cat. number 32106) prior to detection with the iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

Antibody screening by immunoprecipitation on culture media

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 with 30µl 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 ~1 hr at 4°C followed by two washes to remove unbound antibodies.

Starved HAP1 WT media were concentrated as described above and supplemented with protease inhibitor. 0.5 ml aliquots at 1.6 mg/ml of protein were incubated with an antibody-bead conjugate for ~1 hr 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 Western Blot precast midi 4-20% Tris-Glycine polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a concentration of 0.3 µg/ml.

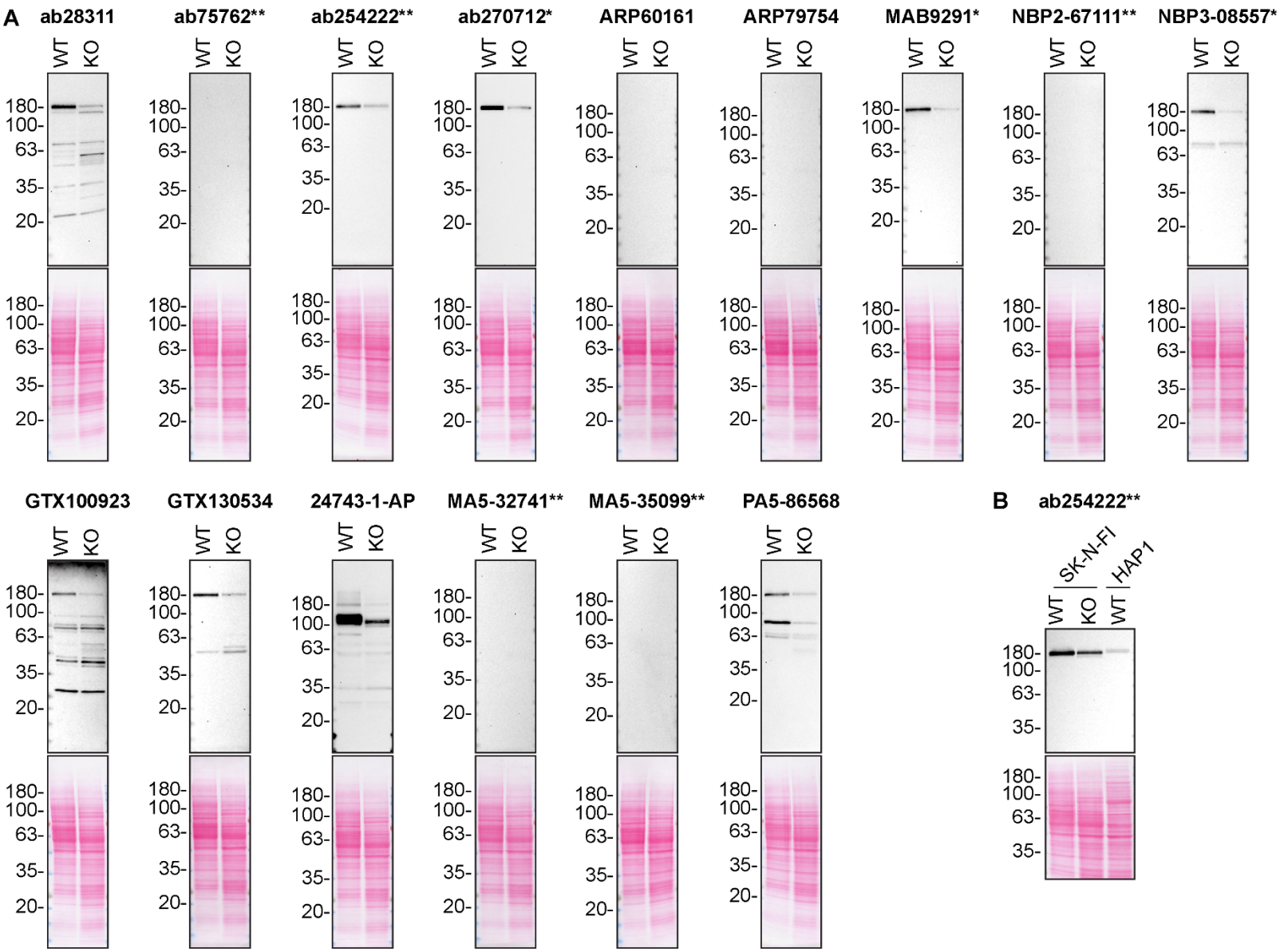

Figure 1: Angiotensin-converting enzyme antibody screening by Western Blot

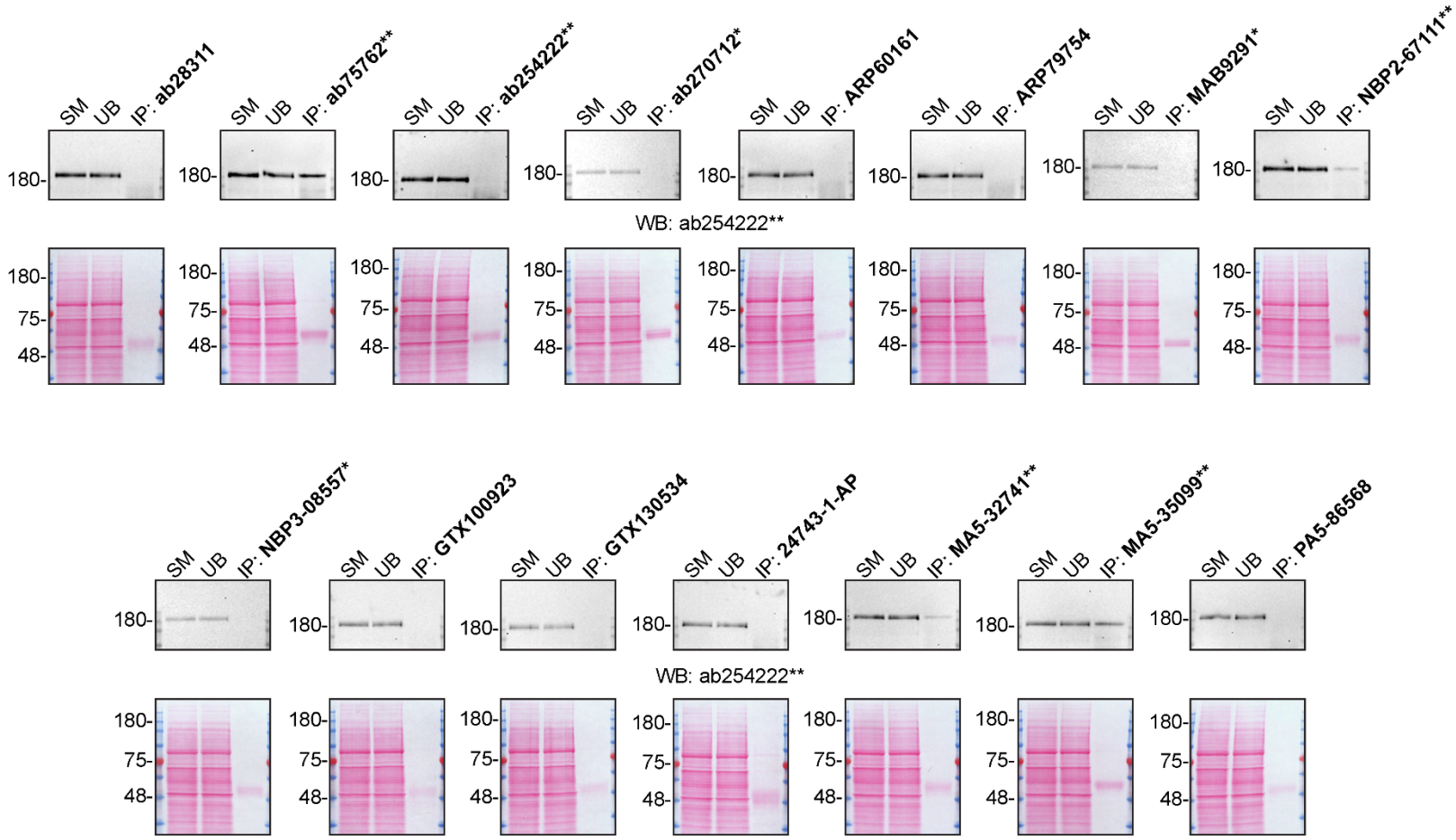

Figure 2: Angiotensin-converting enzyme antibody screening by immunoprecipitation

Figure 1: Angiotensin-converting enzyme antibody screening by Western Blot on culture media.

(A) SK-N-FI WT and *ACE* KO were cultured in serum free media, and 40 µg of protein from concentrated culture media were processed for Western Blot with the indicated Angiotensin-converting enzyme antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: ab28311 at 1/1000, ab75762** at 1/200, ab254222** at 1/10 000, ab270712* at 1/10 000, ARP60161 at 1/200, ARP79754 at 1/200, MAB9291* at 1/500, NBP2-67111** at 1/200, NBP3-08557 at 1/500, GTX100923 at 1/200, GTX130534 at 1/1000, 24743-1-AP at 1/500, MA5-32741** at 1/200, MA5-35099** at 1/200, PA5-86568 at 1/500. **(B)** The level of Angiotensin-converting enzyme in HAP1 is shown. HAP1 WT media were collected and processed as described above. Predicted band size: 150 kDa. *=monoclonal antibody, **=recombinant antibody

Figure 2: Angiotensin-converting enzyme antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on concentrate culture media from HAP1 WT and using 2.0 µg of the indicated Angiotensin-converting enzyme antibodies pre-coupled to Dynabeads protein A or protein G. Samples were washed and processed for Western Blot with the indicated Angiotensin-converting enzyme antibody. For Western Blot, ab254222** was used at 1/2000. The Ponceau stained transfers of each blot are shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate. *=monoclonal antibody, **=recombinant antibody

References

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