Antibody Characterization Report for Apolipoprotein E

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Apolipoprotein E

Short protein name: Apo-E

Gene name: *APOE*

Uniprot: P02649

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 Apolipoprotein E. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Apolipoprotein E by immunoblot (Western blot) and immunoprecipitation. HAP1 was selected based on evidence of appropriate Apolipoprotein E gene expression determined using DepMap [3]. An HAP1 *APOE* KO cell line is available at Horizon Discovery and was used in this study.

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 Apolipoprotein E antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
GeneTex	GTX635889*	44195	AB_2909916	monoclonal	GT27711	mouse	1.0	Wb
GeneTex	GTX635891*	44195	AB_2909917	monoclonal	GT1627	mouse	1.0	Wb
Abcam	ab52607**	GR33789797	AB_867704	recombinant-mono	EP1374Y	rabbit	0.1	Wb,IP,IF
Abcam	ab51015**	GR19880919	AB_867703	recombinant-mono	EP1373Y	rabbit	0.14	Wb,IP,IF
Abcam	ab1907*	GR33619625	AB_302669	monoclonal	E6D7	mouse	1.0	IF
Cell Signaling Technology	13366**	4	AB_2798191	recombinant-mono	D7I9N	rabbit	n/a	Wb,IP,IF
Aviva Systems Biology	ARP54283	QC56479-160608	AB_10640958	polyclonal	-	rabbit	0.5	Wb
Thermo Fisher Scientific	701241**	2477346	AB_2532438	recombinant-mono	16H22L18	rabbit	0.5	Wb,IF
Thermo Fisher Scientific	MA5-41148**	XH3670137	AB_2898902	recombinant-mono	SC0536	rabbit	1.0	Wb
Thermo Fisher Scientific	MA5-15852*	XH3669852	AB_11153583	monoclonal	1H4	mouse	n/a	Wb
Bio-Techne	MAB41441*	ZRQ0318021	AB_2289763	monoclonal	395004	rat	5.0	Wb
Bio-Techne	NB110-60531*	COEN01-2	AB_920623	monoclonal	WUE-4	mouse	1.0	Wb,IP
Proteintech	18254-1-AP	68183	AB_2878525	polyclonal		rabbit	0.4	Wb
Proteintech	66830-1-Ig*	10008911	AB_2882173	monoclonal	1B2C9	mouse	2.1	Wb,IF

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

Table 2: Summary of the cell lines used

Institution	Catalog number	RRID (Cellosaurus)	Cell line	genotype
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC005366c001	CVCL_SC97	HAP1	*APOE* KO

Figure 1: Apolipoprotein E antibody screening by immunoblot on culture media.

HAP1 WT and *APOE KO* were cultured in serum free media. Media was collected and concentrated. Then, 30 µg of protein from concentrated culture media were processed for immunoblot with the indicated Apolipoprotein E antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: GTX635889* at 1/200, GTX635891* at 1/200, ab52607** at 1/1000, ab51015** at 1/1000, ab1907* at 1/1000, 13366** at 1/500, ARP54283 at 1/1000, 701241** at 1/200, MA5-41148** at 1/1000, MA5-15852* at 1/1000, MAB41441* at 1/200, NB110-60531* at 1/200, 18254-1-AP at 1/200, 66830-1-Ig* at 1/200. Apolipoprotein E predicted band size: 36 kDa. *=monoclonal antibody, **=recombinant antibody.

Figure 2: Apolipoprotein E antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on 0.9 mg of concentrated culture media using 2.0 µg of the indicated Apolipoprotein E antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Apolipoprotein E antibodies. For immunoblot, 13366** was used at 1/500. The Ponceau stained transfers of each blot are shown. SM=3% starting material; UB=3% unbound fraction; IP=immunoprecipitate; HC=heavy chain; *=monoclonal antibody, **=recombinant antibody.

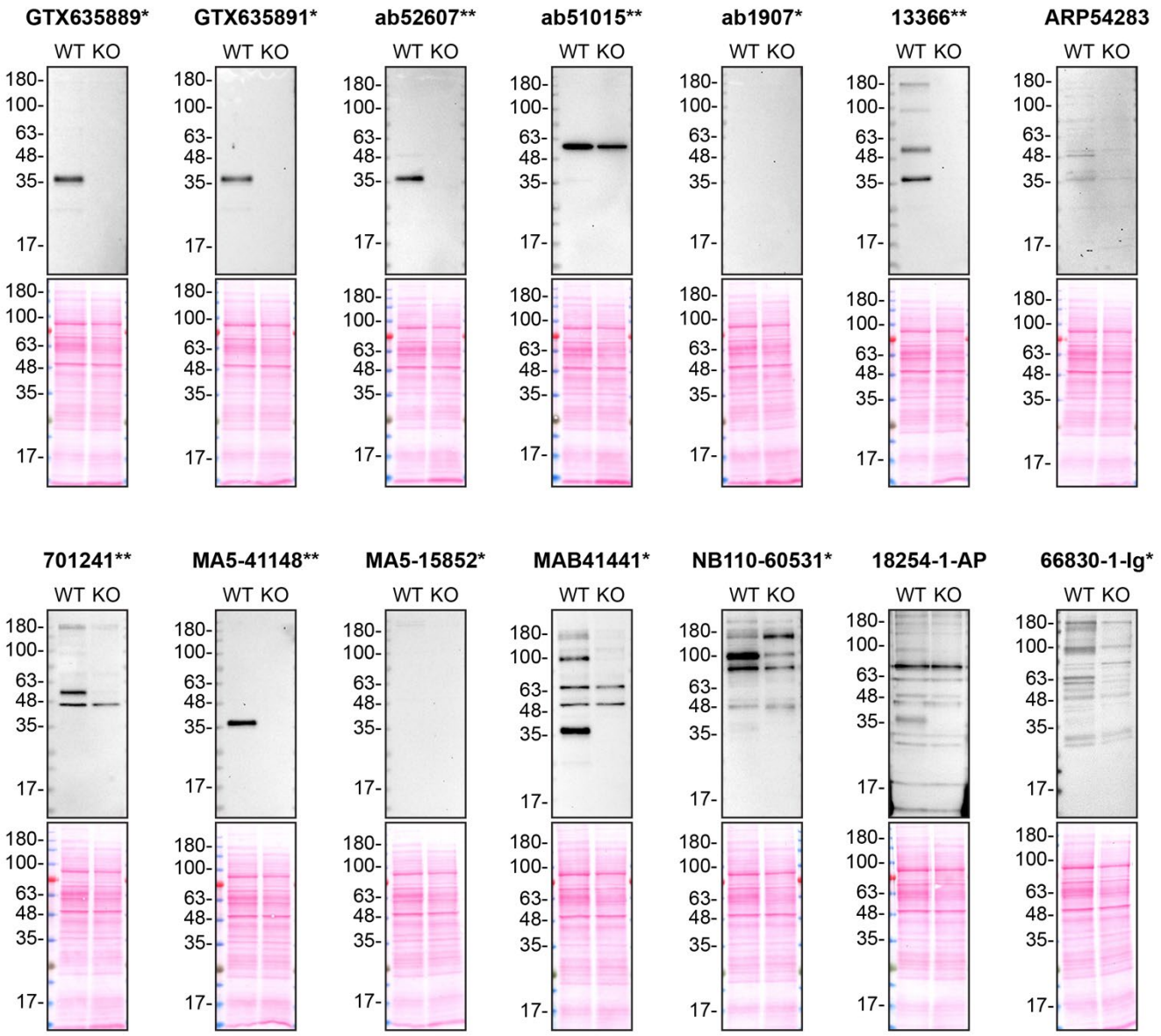

Figure 1: Apolipoprotein E antibody screening by immunoblot on culture media

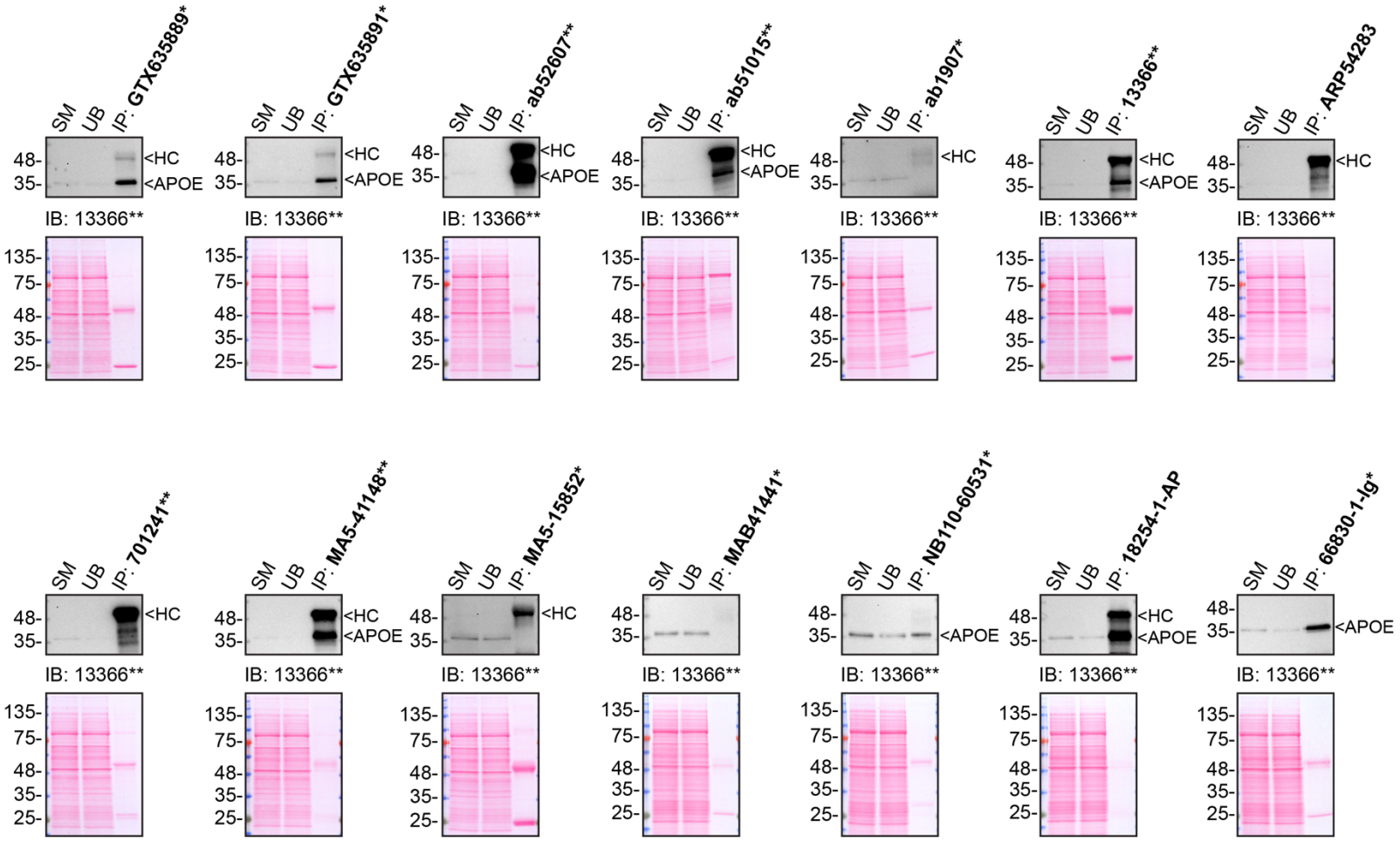

Figure 2: Apolipoprotein E antibody screening by immunoprecipitation on culture media

Materials and methods

Antibodies

All tested Apolipoprotein E antibodies are listed in Table 1. Peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies are from Thermo Fisher Scientific (cat. number 62-6520 and 65-6120).

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.

Collection of culture media

HAP1 WT and *APOE* KO cells were washed 3x with PBS 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 10kDa (MilliporeSigma cat. number UFC901024).

Antibody screening by immunoblot using culture media

Immunoblots were performed as described in our standard operating procedure [4]. Midi precast 4-20% gradient polyacrylamide gels from Thermo Fisher Scientific (cat. Number WXP42012BOX) were used and transferred on nitrocellulose membranes. Proteins were visualized on the membranes with Ponceau staining which is scanned to show together with individual immunoblots. 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 Pierce ECL from Thermo Fisher Scientific (cat. number 32106) or with Clarity Western ECL Substrate from Bio-Rad (cat. number 1705061) prior to detection with the iBright™ CL1500 Imaging System from Thermo Fisher Scientific (cat. number A44240).

Antibody screening by immunoprecipitation using culture media

Immunoprecipitation was performed as described in our SOP for immunoprecipitation [5]. Antibody-bead conjugates were prepared by adding 2 µg or 20 µl of an antibody at an unknown concentration to 500 µl of Pierce IP Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30µl of Dynabeads protein A from Thermo Fisher Scientific (cat. number 10002D). Pierce IP Lysis Buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) was supplemented with the Halt Protease Inhibitor Cocktail 100X from Thermo Fisher Scientific (cat. number 78446) at a final concentration of 1x. Tubes were rocked for ~1 hr at 4°C followed by several washes to remove unbound antibodies.

Starved HAP1 WT culture media were concentrated as described above. 0.6 ml aliquots at 1.5 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 IP Lysis Buffer and processed for SDS-PAGE and immunoblot on midi precast 4-20% polyacrylamide gels. Prot-A:HRP (MilliporeSigma, cat. number P8651) was used as a secondary detection system at a concentration of 0.4 µg/ml.

References

1. Laflamme, C., et al., *Opinion: Independent third-party entities as a model for validation of commercial antibodies.* N Biotechnol, 2021. **65**: p. 1-8 DOI: 10.1016/j.nbt.2021.07.001.
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