Antibody Characterization Report for Midkine

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Midkine

Alternative protein names: MK, Amphiregulin-associated protein, ARAP, Midgestation and kidney protein, Neurite outgrowth-promoting factor 2, Neurite outgrowth-promoting protein

Gene name: *MDK*

Uniprot: P21741

This report guides researchers to select the most appropriate antibodies for Midkine. We used an antibody characterization pipeline [1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Midkine. Midkine is a secreted protein [2] and thus we tested antibodies by immunoblot (Western blot) and immunoprecipitation on total cell lysates and on serum-free culture media. HAP1 was selected based on evidence of appropriate Midkine expression determined through DepMap [3], a public expression database. An HAP1 *MDK* KO line is available at Horizon Discovery.

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.

1. Laflamme, C., et al., *Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72.* Elife, 2019. **8** DOI: 10.7554/eLife.48363.
2. Tomomura, M., et al., *A retinoic acid-responsive gene, MK, found in the teratocarcinoma system. Heterogeneity of the transcript and the nature of the translation product.* J Biol Chem, 1990. **265**(18): p. 10765-70.
3. *DepMap, Broad*. 2019.

Table 1: Summary of the Midkine antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
GeneTex	GTX108439	39855	AB_1950903	polyclonal	-	rabbit	0.33	Wb
GeneTex	GTX116089	40597	AB_11165850	polyclonal	-	rabbit	1.00	Wb
Bio-Techne	AF-258-PB	WE0519091	AB_2143400	polyclonal	-	goat	0.20	Wb
Bio-Techne	MAB2582	CMHR0219071	AB_2893288	recombinant-mono	1011522	mouse	0.50	IF
Bio-Techne	MAB2583	CMLT0120031	AB_2893289	monoclonal	1011622	mouse	0.50	other
Bio-Techne	NBP2-66948	HN0907	AB_2893290	recombinant-mono	JF096-5	rabbit	1.00	Wb, IP
Thermo	MA5-32538	WD3265256	AB_2809815	recombinant-mono	JF096-5	rabbit	1.00	Wb
Abcam	ab52637	GR3315059-2	AB_880698	recombinant-mono	EP1143Y	rabbit	0.10	Wb, IP, IF

Wb=Western blot; IP= immunoprecipitation; IF=immunofluorescence

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	HZGHC007906c008	-	HAP1	*MDK* KO

Figure 1: Inhibition of Midkine secretion by Brefeldin A.

HAP1 cells (WT and *MDK* KO) were treated with Brefeldin A at 3.0 µg/ml for 18 hrs. Lysates of treated and non-treated HAP1 cells were prepared, and 50 µg of protein were processed for immunoblot with the indicated Midkine antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: GTX116089 at 1/500; AF-258-PB at 1/100; MA5-32538 at 1/500; ab52637 at 1/500. Predicted band size: 15 kDa.

Figure 2: Midkine antibody screening by immunoblot on culture media.

A) Schematic of the protocol used to concentrate culture media. **B)** ~30 µg of protein from concentrated culture media were processed for immunoblot with the indicated Midkine antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: GTX108439 at 1/1000; GTX116089 at 1/100; AF-258-PB at 1/100; NBP2-66948 at 1/500; MAB2582 at 1/200; MAB2583 at 1/200; MA5-32538 at 1/500; ab52637 at 1/500. Predicted band size: 15 kDa.

Figure 3: Midkine antibody screening by immunoprecipitation on culture media.

Concentrated culture media was prepared as in 2A). Immunoprecipitation was performed on concentrated culture media using 1.0 µg of the indicated Midkine antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Midkine antibodies. For immunoblot, AF-258-PB was used at 1/500, NBP2-66948 at 1/500, MA5-32538 at 1/200 and ab52637 at 1/200. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain.

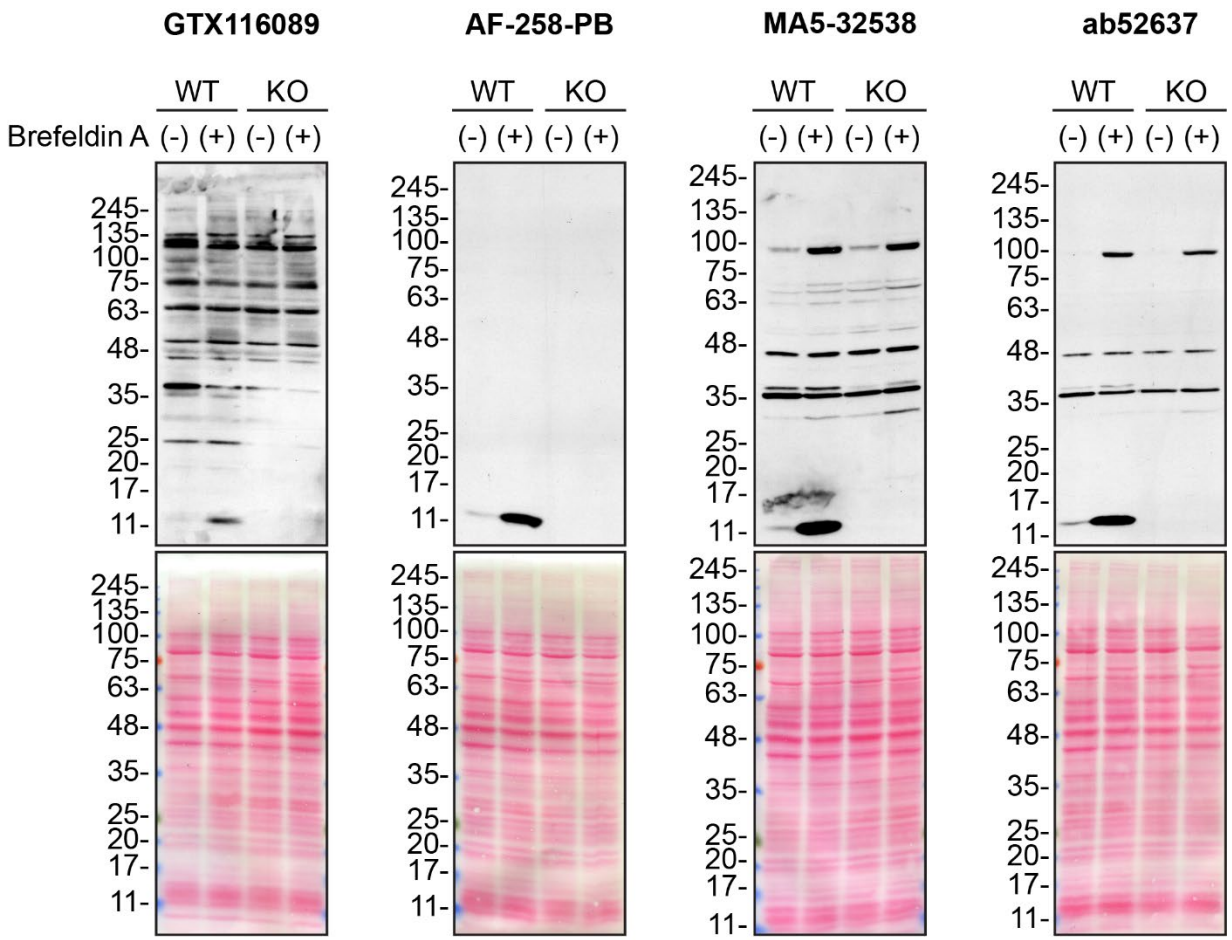

Figure 1: Inhibition of Midkine secretion by Brefeldin A

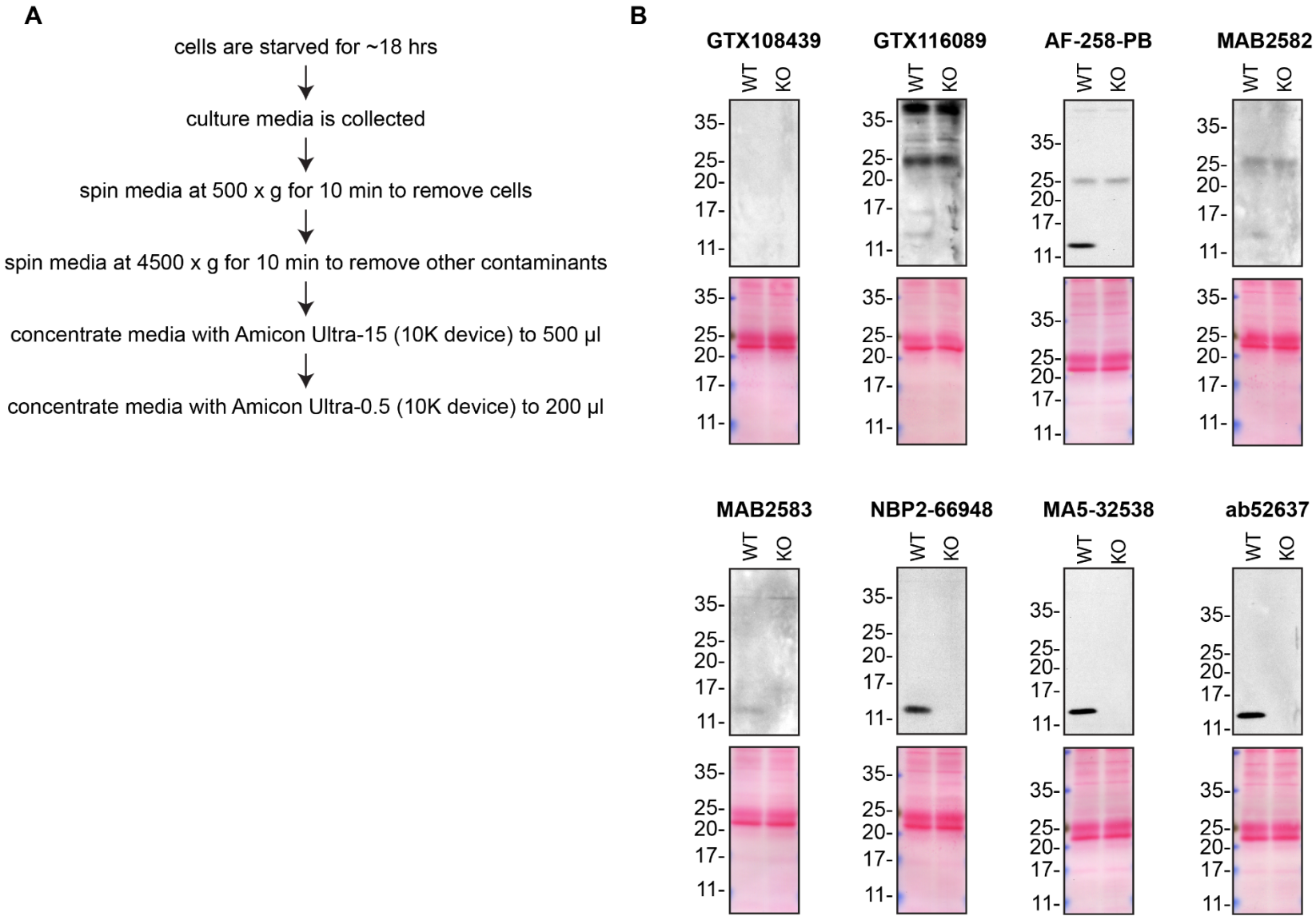

Figure 2: Midkine antibody screening by immunoblot on culture media

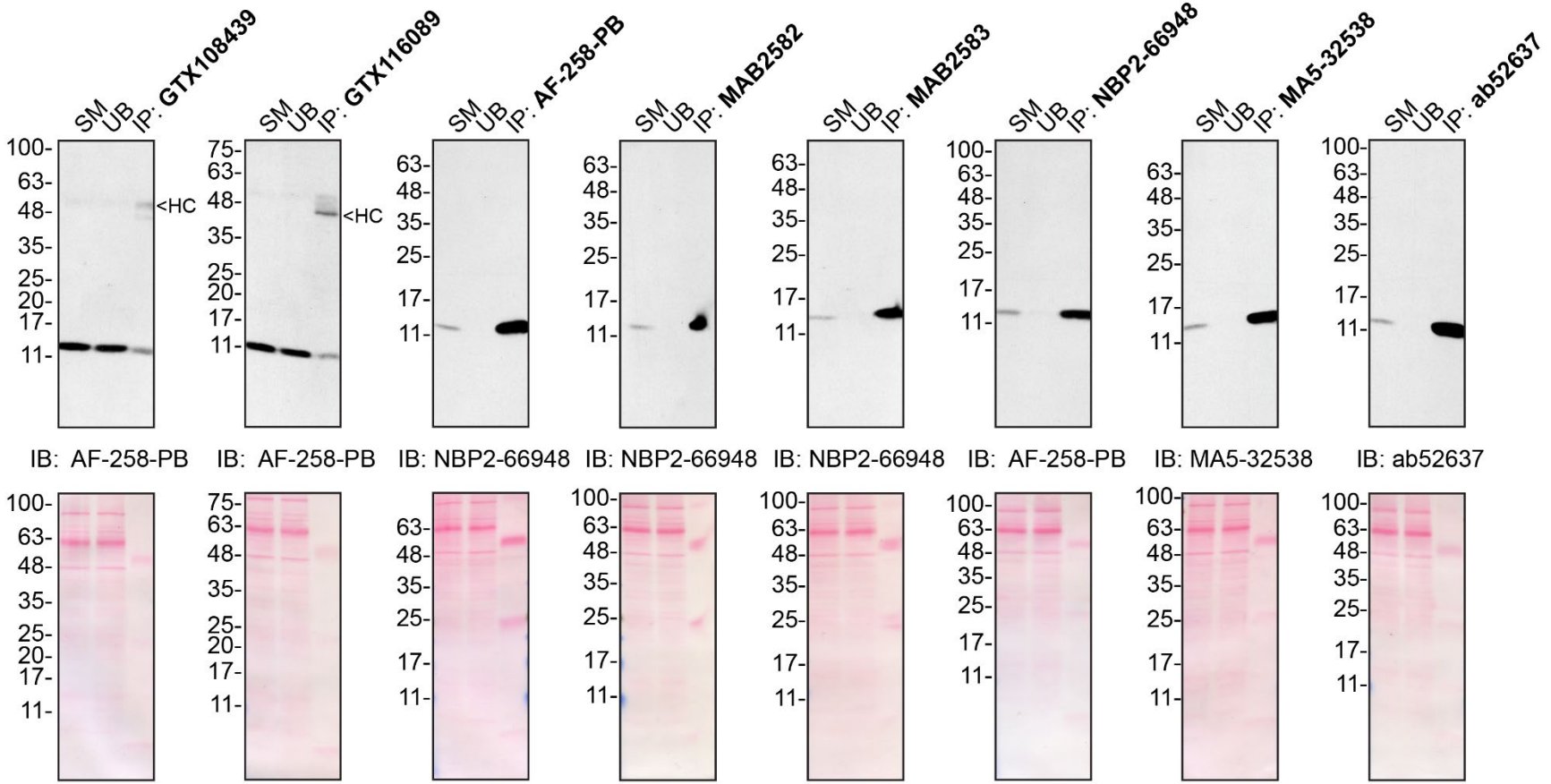

Figure 3: Midkine antibody screening by immunoprecipitation on culture media

Materials and methods

Antibodies

All Midkine 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

HAP1 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.

Lysates from Brefeldin A-treated cells

HAP1 were treated with 0.3 µg/ml of Brefeldin A from Thermo Fisher Scientific (cat. number 00-4506-51). HAP1 (WT and *MDK* KO) treated and non-treated 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. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

Immunoblots were performed with large 10-20% 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).

Antibody screening by immunoblot

HAP1 cells (WT and *MDK* KO) were washed 3x with PBS and starved for ~18 hrs as described above. 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 initially concentrated using Amicon Ultra-15 Centrifugal Filter Units (MilliporeSigma cat. number UFC9010) by centrifuging at 4000 x g for 15min. The resulting 500 µl of the concentrated media were centrifuged again at 4000 x g for 15min using Amicon Ultra-0.5 Centrifugal Filter Units (MilliporeSigma cat. number UFC5010) to 200 µl.

Immunoblots were performed as described above.

Antibody screening by immunoprecipitation

Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 µl of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together 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). Pierce IP Lysis Buffer was supplemented with the Halt Protease and Phosphatase Inhibitor Cocktail 100X from Thermo Fisher Scientific (cat. number 78446) at a final concentration of 1x. Tubes were rocked for ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

Starved HAP1 WT culture media were concentrated as described above. Concentrated culture media were diluted in Pierce IP Lysis Buffer, and 1ml aliquots at 0.3 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 on 10-20% polyacrylamide gels. Prot-A: HRP was used as a secondary detection system (MilliporeSigma, cat. number P8651) at a dilution of 0.4 µg/ml for an experiment where a rabbit antibody was used for both immunoprecipitation and its corresponding immunoblot.