

Antibody Characterization Report for Serine protease HTRA1

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Serine protease HTRA1

Alternative protein names: High-temperature requirement A serine peptidase 1, Serine protease 11, L56

Gene name: *HTRA1*

Uniprot: Q92743

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 Serine protease HTRA1. We used an antibody characterization pipeline [2] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Serine protease HTRA1 by immunoblot (Western blot) and immunoprecipitation. HAP1 was selected based on evidence of appropriate Serine protease HTRA1 gene expression determined using DepMap [3]. An HAP1 *HTRA1* 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 Serine *protease HTRA1* antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Thermo Fisher Scientific	PA5-11412	XF3612574	AB_2122815	polyclonal	-	rabbit	0.50	Wb,IF
Thermo Fisher Scientific	PA5-83344	XF3611767	AB_2790500	polyclonal	-	rabbit	0.10	IF
Thermo Fisher Scientific	PA5-115386	XB3491406	AB_2900022	polyclonal	-	rabbit	1.00	Wb,IF
Abcam	ab274322**	GR3414793	AB_2938804	recombinant-mono	EPR23240-64	rabbit	0.56	Wb,IP
Proteintech	55011-1-AP	36017	AB_10859830	polyclonal	-	rabbit	0.85	Wb,IP,IF
ABclonal	A11693	84370201	AB_2758692	polyclonal	-	rabbit	1.47	Wb

Wb=Western blot, IP= immunoprecipitation, IF=immunofluorescence, **=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	HZGHC007986c006	CVCL_C1LH	HAP1	<i>HTRA1</i> KO

Figure 1: Serine protease HTRA1 antibody screening by immunoblot on culture media.

HAP1 WT and *HTRA1 KO* were cultured in serum free media. Media was collected and concentrated. Then, 25 µg of protein from concentrated culture media were processed for immunoblot with the indicated Serine protease HTRA1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used: PA5-11412 at 1/1000, PA5-83344 at 1/500, PA5-115386 at 1/500, ab274322** at 1/1000, 55011-1-AP at 1/500, A11693 at 1/1000. Serine protease HTRA1 predicted band size: 51 kDa. **=recombinant antibody.

Figure 2: Serine protease HTRA1 antibody screening by immunoprecipitation on culture media.

Immunoprecipitation was performed on 1 mg of concentrated culture media using 2.0 µg of the indicated Serine protease HTRA1 antibodies pre-coupled to either protein G or protein A magnetic beads. Samples were washed and processed for immunoblot with the indicated Serine protease HTRA1 antibodies. For immunoblot, PA5-11412 was used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=2% starting material; UB=2% unbound fraction; IP=immunoprecipitated; **=recombinant antibody.

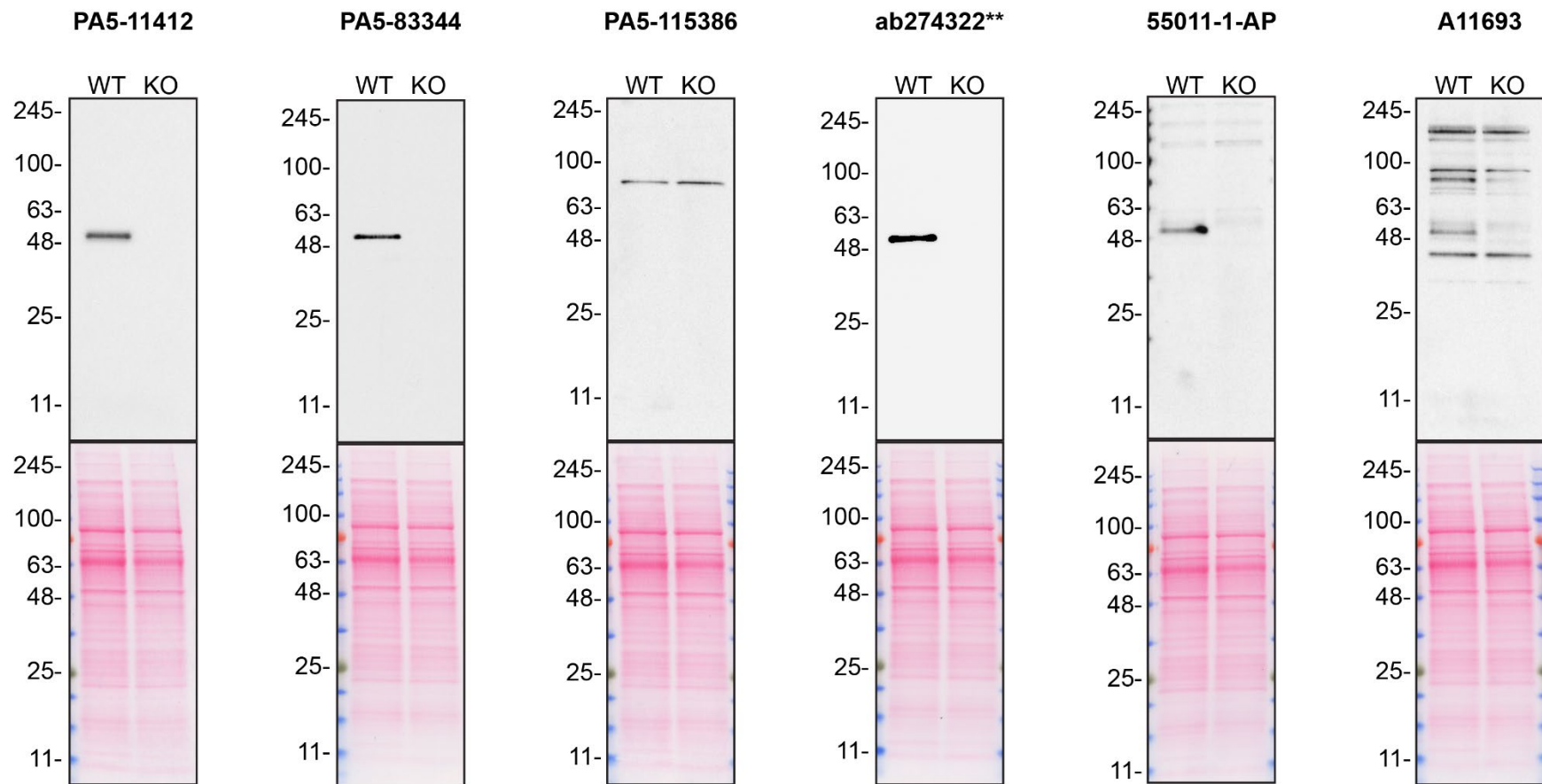


Figure 1: Serine protease HTRA1 antibody screening by immunoblot on culture media

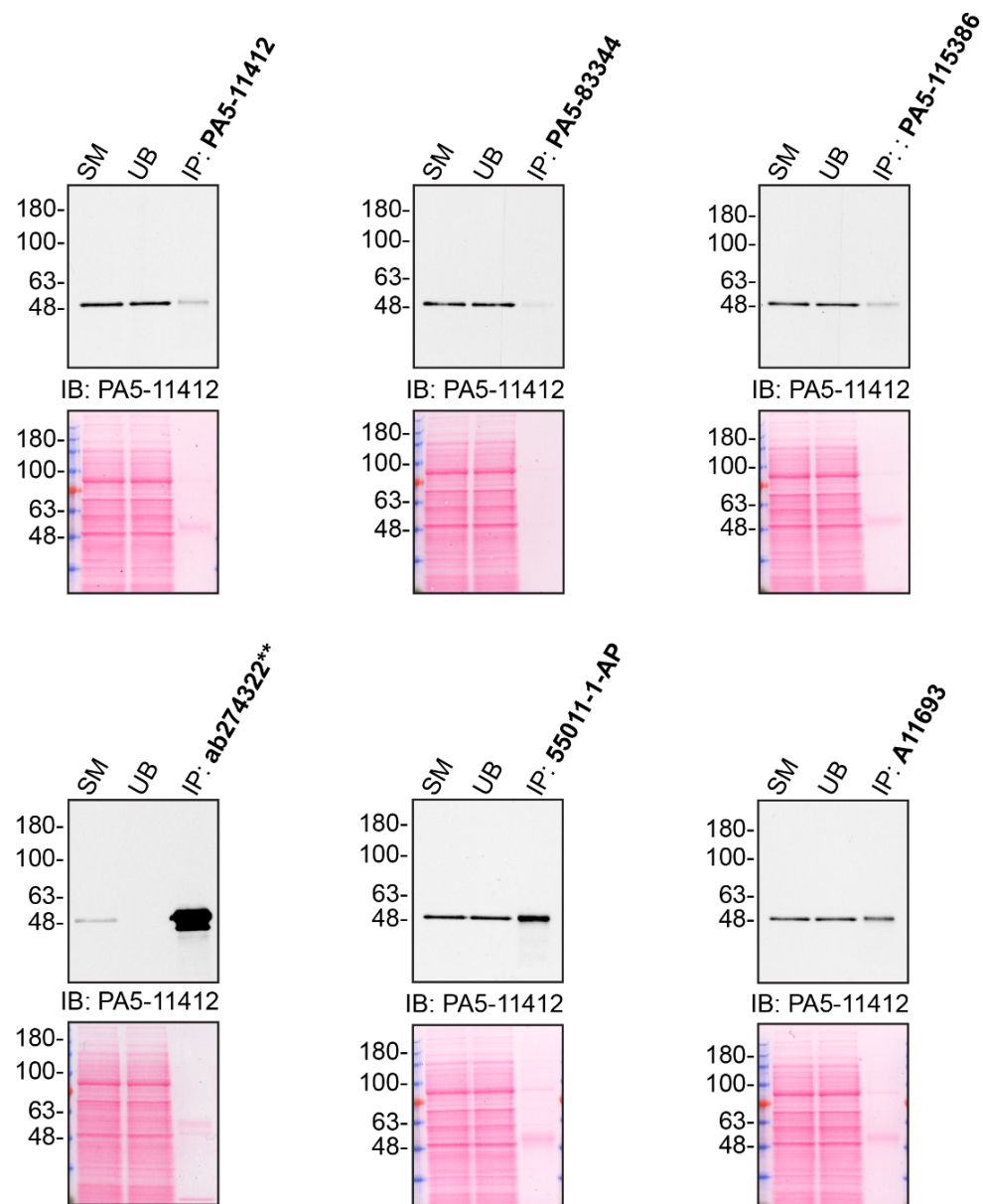


Figure 2: Serine protease HTRA1 antibody screening by immunoprecipitation on culture media

Materials and methods

Antibodies

All Serine protease HTRA1 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 *HTRA1* 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 [5]. 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 ECL from Pierce (cat. number 32106) prior to detection with HyBlot CL autoradiography films from Denville (cat. number 1159T41).

Antibody screening by immunoprecipitation using culture media

Immunoprecipitation was performed as described in our SOP for immunoprecipitation [6]. Antibody-bead conjugates were prepared by adding 2 µg of antibody to 500 µl of Pierce IP Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30 µl 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 ~2 hrs at 4°C followed by several washes to remove unbound antibodies.

Starved HAP1 WT culture media were concentrated as described above. 1ml aliquots at 1 mg/ml of protein 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 IP Lysis Buffer and processed for SDS-PAGE and immunoblot on midi precast 4-20% polyacrylamide gels.

References

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