



Antibody Characterization Report for Peroxiredoxin-1

YCharOS Antibody Characterization Report

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Target:

Protein name: Peroxiredoxin-1

Alternative protein names: PAG, NKEF-A

Uniprot: Q06830

Gene name: *PRDX1*

Alternative gene names: *PAGA, PAGB, TDPX2*

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Table 1: Summary of the Peroxiredoxin-1 antibodies tested

| Company | Catalog number | Lot number | RRID | Clonality | Clone ID | Host | Concentration (µg/µl) |
|----------------|-----------------------|-------------------|-------------|------------------|-----------------|-------------|------------------------------|
| Proteintech | 15816-1-AP | not provided | AB_2170318 | polyclonal | - | rabbit | 0.14 |
| Proteintech | 66820-1-Ig | not provided | AB_2882163 | monoclonal | 2B2A2 | mouse | 1.0 |
| Thermo | LF-MA0073 | VJ3101923 | AB_1069882 | monoclonal | 6E5 | mouse | not provided |
| Thermo | PA3-750 | VA299223 | AB_2168166 | polyclonal | - | rabbit | not provided |
| Bio-Techne | AF3488 | XLO0318011 | AB_2170320 | polyclonal | - | goat | 0.2 |
| Bio-Techne | MAB3488 | CDBP0316021 | AB_2170319 | monoclonal | 477609 | mouse | 0.5 |
| GeneTex | GTX101705 | 39577 | AB_1241175 | polyclonal | - | rabbit | 10.0 |
| GeneTex | GTX113793 | 40135 | AB_2037651 | polyclonal | - | rabbit | 0.57 |
| Abcam | ab41906 | GR3226661-2 | AB_2284360 | polyclonal | - | rabbit | 1.0 |
| Abcam | ab109498 | GR48348-14 | AB_10865914 | recombinant-mono | EPR5433 | rabbit | 0.18 |
| Abcam | ab109506 | GR-43632-12 | AB_10864539 | recombinant-mono | EPR5434 | rabbit | 0.05 |
| Santa-Cruz | sc-59656 | H1720 | AB_2168191 | monoclonal | 2A4 | mouse | not provided |

Table 2: Summary of the cell lines used

| Institution | Catalog number | RRID (Cellosaurus) | Cell line | genotype |
|---------------------------------|-----------------------|---------------------------|------------------|-----------------|
| Montreal Neurological Institute | - | CVCL_0042 | U2OS | WT |
| Montreal Neurological Institute | - | CVCL_A4GN | U2OS | <i>PRDX1</i> KO |

Figure 1: Analysis of Peroxiredoxin-1 antibodies by immunoblot.

Lysates of U2OS (WT and *PRDX1* KO) were prepared and 10 µg of lysates were processed for immunoblot with the indicated Peroxiredoxin-1 antibodies. The Ponceau stained transfers associated with each blot are shown. Antibody dilution used: 15816-1-AP at 1/5000; 66820-1-Ig at 1/5000; LF-MA0073 at 1/5000; PA3-750 at 1/1000; AF3488 at 1/1000; MAB3488 at 1/1000; GTX101705 at 1/1000; GTX113793 at 1/1000; ab41906 at 1/1000; ab109498 at 1/5000; ab109506 at 1/5000; sc-59656 at 1/1000.

Figure 2: Analysis of Peroxiredoxin-1 antibodies by immunoprecipitation.

Lysates were prepared and IP was performed using 1.0 µg of the indicated Peroxiredoxin-1 antibodies pre-coupled to either protein G or protein A Sepharose beads. The antibody concentration for both LF-MA0073 and PA3-750 is not provided and 1.0 µl was used for the IP. Samples were washed and processed for immunoblot with the indicated Peroxiredoxin-1 antibody. For immunoblot, AF3488 and MAB3488 were used at 1/10000 and 1/5000, respectively. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain; LC= antibody light chain.

Figure 3: Analysis of Peroxiredoxin-1 antibodies by immunofluorescence.

Parental and *PRDX1* KO cells were transfected with a GFP or mCherry plasmid, respectively. Parental and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Peroxiredoxin-1 antibodies and with the corresponding Alexa-fluor 647 coupled secondary antibody. Acquisition of the green (parental), red (KO) and far-red (antibody staining) channels was performed. Parental and KO cells are outlined with green and red dashed line, respectively. Grayscale images of the far-red channel are shown. Representative images are shown. Bars = 40 µm. Antibody dilution used: 15816-1-AP at 1/500; 66820-1-Ig at 1/1000; LF-MA0073 at 1/1000; PA3-750 at 1/1000; AF3488 at 1/500; MAB3488 at 1/50000; sc-59656 at 1/10000; GTX101705 at 1/1000; GTX113793 at 1/1000; ab41906 at 1/1000; ab109498 at 1/500; ab109506 at 1/250.

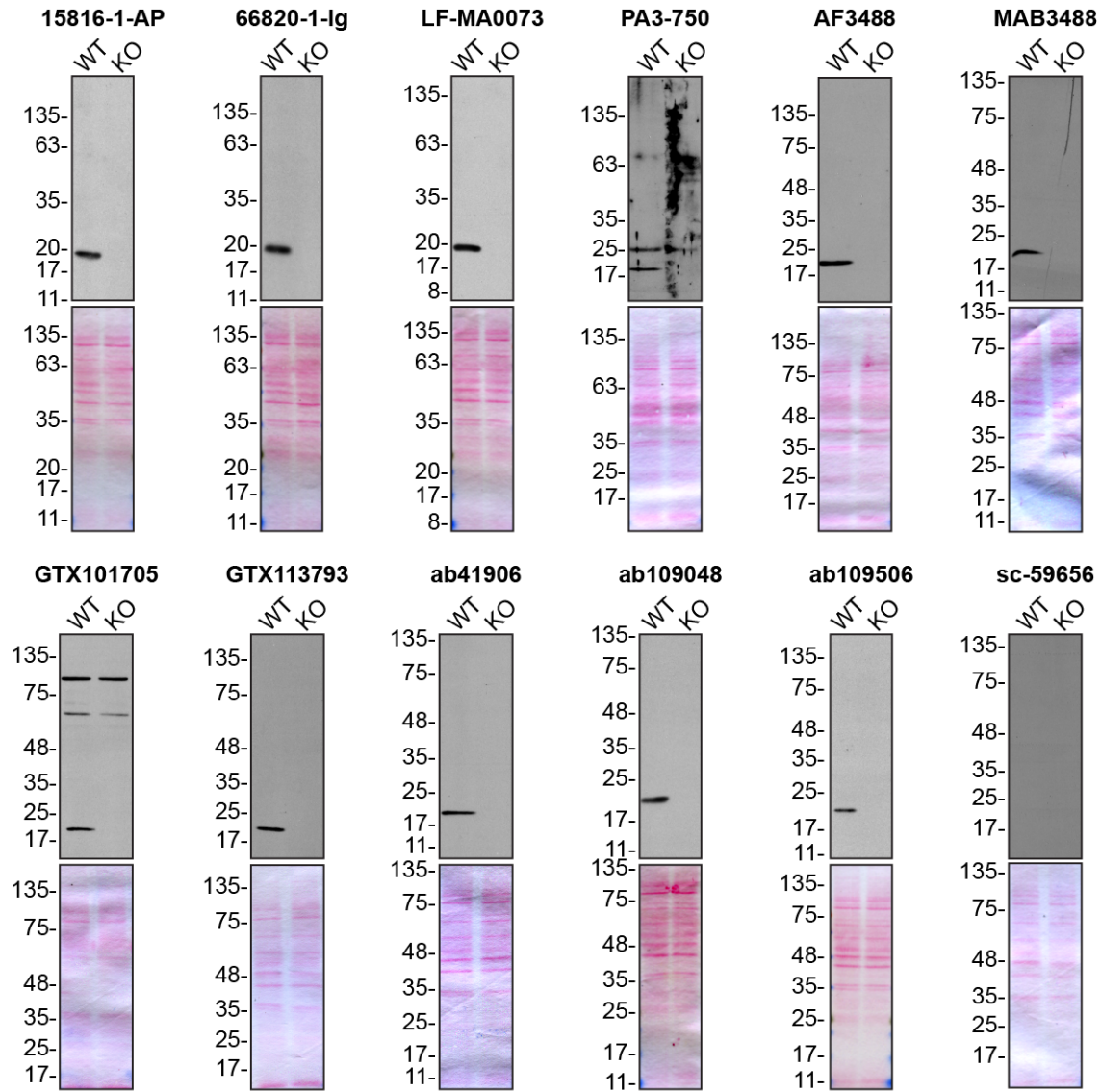


Figure 1: Peroxiredoxin-1 antibody screening by immunoblot

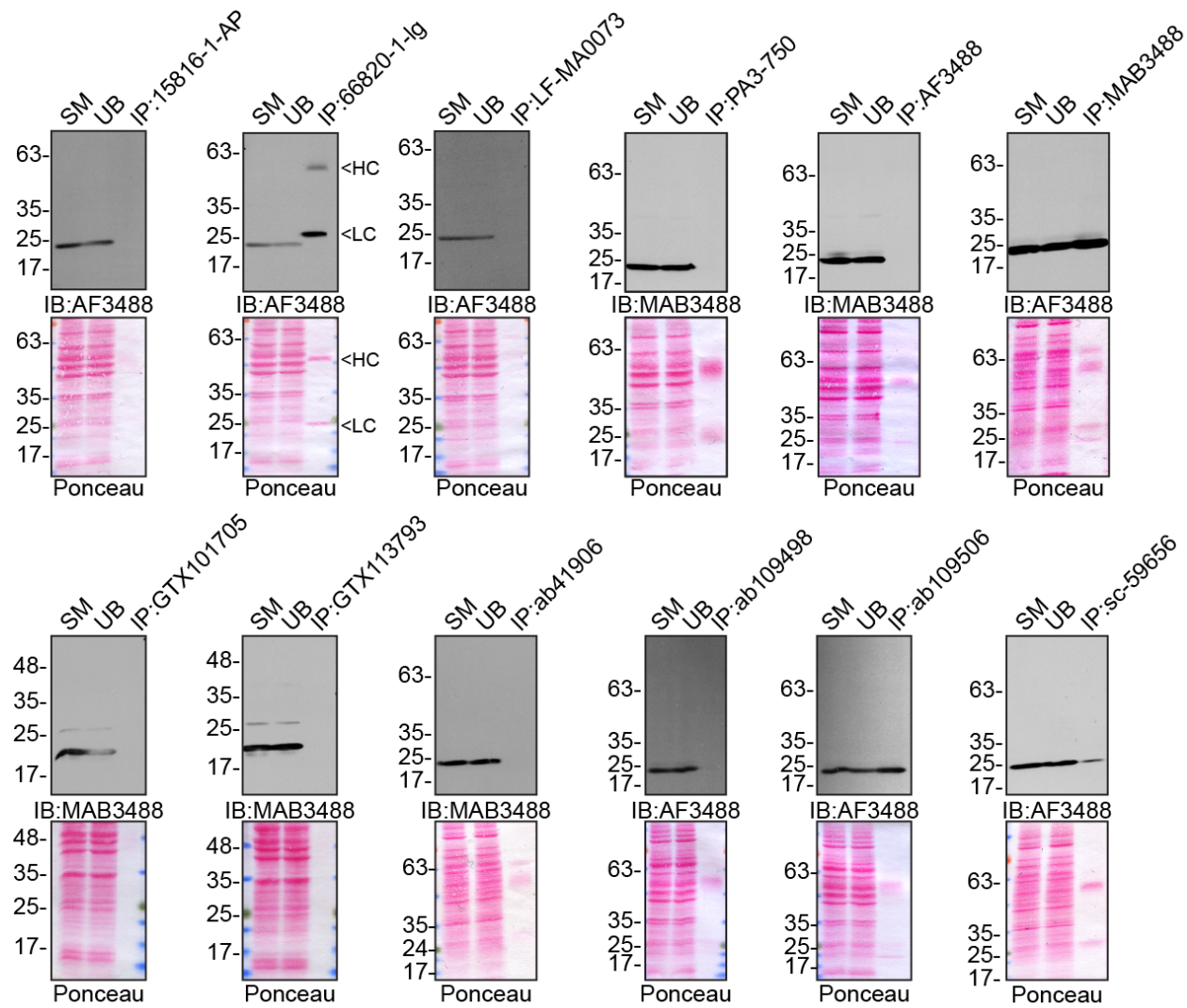


Figure 2: Peroxiredoxin-1 antibody screening by immunoprecipitation

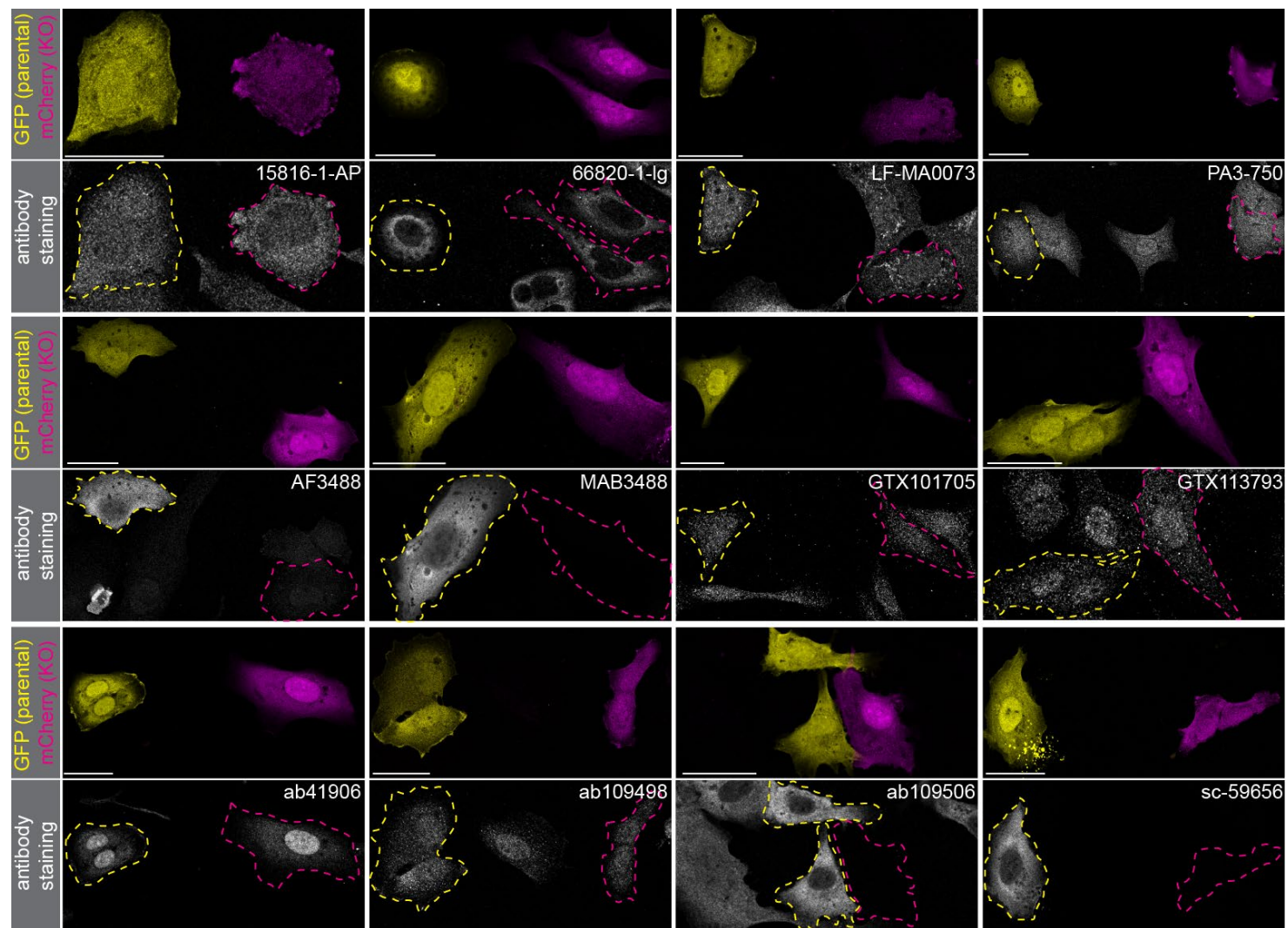


Figure 3: Peroxiredoxin-1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Peroxiredoxin-1 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). Alexa-647-conjugated goat anti-mouse, goat anti-rabbit and donkey anti-goat secondary antibodies are from Thermo Fisher Scientific (cat. number A21236, A21245 and A21447).

Cell lines

U2OS *PRDX1* KO clone was generated by following an open-access protocol available on Zenodo.org: <https://zenodo.org/record/3875777#.X9uE11VKjIX>. The gRNA sequence used to introduce a STOP codon in the *PRDX1* gene is: cttgttgaccactgtgtga.

U2OS WT and KO were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% bovine calf serum (GE Healthcare cat. number SH30072.03), 2 mM L-glutamate (Wisent cat. number 609065, 100 IU penicillin and 100 µg/ml streptomycin (Wisent cat. number 450201).

Immunoblot

U2OS parental and *PRDX1* KO cells 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 ~240,000xg for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and immunoblot.

Immunoblots were performed with large 4-15% 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).

Immunoprecipitation

Antibody-bead conjugate were prepared by adding 1.0 µg of antibody to 500 µl of PBS with 0,01% triton X-100 in a microcentrifuge tube, together with 30 µl of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) Sepharose beads. Tubes were rocked O/N at 4°C followed by several washes to remove unbound antibodies.

U2OS were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) supplemented with protease inhibitor. Following 30 min on ice, lysates were spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1 mg/ml of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. Following centrifugation, the unbound fractions were collected and beads were subsequently washed three times with 1 ml of HEPES lysis buffer, and processed for SDS-PAGE and immunoblot.

Immunofluorescence

U2OS cells (parental and *PRDX1* KO) were transfected with GFP and mCherry respectively. At 48 hrs post transfection, both cell lines were plated on glass coverslips as a mosaic and incubated for 24 hrs. 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 15 min at room temperature and blocked in PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Coverslips were incubated face down on a 50 µl drop (on paraffin film in a moist chamber) of IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Peroxiredoxin-1 antibodies O/N at 4°C. Cells were washed 3 × 10 min and incubated with corresponding Alexa Fluor 647-conjugated secondary antibodies diluted 1:2000 in IF buffer for 1 hr at room temperature. Cells were washed 3 × 10 min with IF buffer and once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Leica SP8 laser scanning confocal microscope equipped with a 40x oil objective (NA = 1.30) and HyD detectors. Acquisition was performed using Leica Application Suite X software (version 3.1.5.16308). Analysis was done using Image J. All cell images represent a single focal plane. Figures were prepared for publication using Adobe Photoshop to adjust contrast, apply 1 pixel Gaussian blur and then assembled with Adobe Illustrator.