Antibody Characterization Report for Serine/threonine-protein kinase Nek1

YCharOS Antibody Characterization Report

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Target:

Recommended protein name: Serine/threonine-protein kinase Nek1

Protein name (short): Nek1

Alternative protein names: Never in mitosis A-related kinase 1, NimA-related protein kinase 1, Renal carcinoma antigen NY-REN-55

Gene name: *NEK1*

Uniprot: Q96PY6

This report guides researchers to select the most appropriate antibodies for Nek1. We used an antibody characterization pipeline[1] based on knockout (KO) cells to perform head-to-head comparisons of commercial antibodies for Nek1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. HeLa was selected based on evidence of appropriate Nek1 protein expression determined through public proteomics databases, namely PaxDB[2] and DepMap[3]. HeLa was modified with CRISPR/Cas9 to knockout[4] the corresponding *NEK1* gene.

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* **8**, doi:10.7554/eLife.48363 (2019).

2 Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. & von Mering, C. Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163-3168, doi:10.1002/pmic.201400441 (2015).

3 Nusinow, D. P. *et al.* Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell* **180**, 387-402 e316, doi:10.1016/j.cell.2019.12.023 (2020).

4 Shlaifer, I. *et al. Generation of Knockout Cell Lines Using CRISPR-Cas9 Technology*, <https://zenodo.org/record/3738361#.YIyeDu2SlaR> (February 24, 2020).

Table 1: Summary of the Nek1 antibodies tested

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µl)	Vendors recommended applications
Proteintech	27146-1-AP	00054983	AB_2880773	polyclonal	-	rabbit	0.33	Wb, IP
Abcam	ab229489	GR329661-2	AB_2885033	polyclonal	-	rabbit	0.33	Wb, IF
GeneTex	GTX130828	42375	AB_2886357	polyclonal	-	rabbit	0.33	Wb, IF
Thermo	PA5-78074	VL3152096D	AB_2736203	polyclonal	-	rabbit	0.33	Wb, IF
Thermo	PA5-54271	VL3152387B	AB_2644600	polyclonal	-	rabbit	0.70	-
Santa-Cruz	sc-398813	C1218	AB_2885034	monoclonal	E-10	mouse	0.20	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
ATCC	CCL-2	CVCL_0030	HeLa	WT
Montreal Neurological Institute	-	CVCL_A8DV	HeLa	*NEK1* KO

Figure 1: Nek1 antibody screening by immunoblot.

Lysates of HeLa (WT and *NEK1* KO) were prepared and 100 µg of protein were processed for immunoblot with the indicated Nek1 antibodies. The Ponceau stained transfers of each blot are shown. Antibody dilution used was 1/1000, except for sc-398813 which was used at 1/250. Predicted band size: ~143 kDa.

Figure 2: Nek1 antibody screening by immunoprecipitation.

HeLa lysates were prepared and immunoprecipitation was performed using 1.0 µg of the indicated Nek1 antibodies pre-coupled to either protein G or protein A Sepharose beads. Samples were washed and processed for immunoblot with the indicated Nek1 antibody. For immunoblot, PA5-78074 and GTX130828 were used at 1/1000. The Ponceau stained transfers of each blot are shown. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate.

Figure 3: Nek1 antibody screening by immunofluorescence.

HeLa WT and *NEK1* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated Nek1 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilution used: 27146-1-AP at 1/300; ab229489 at 1/300; GTX130828 at 1/300; PA5-78074 at 1/300; PA5-54271 at 1/700; sc-398813 at 1/300. Bars = 10 µm.

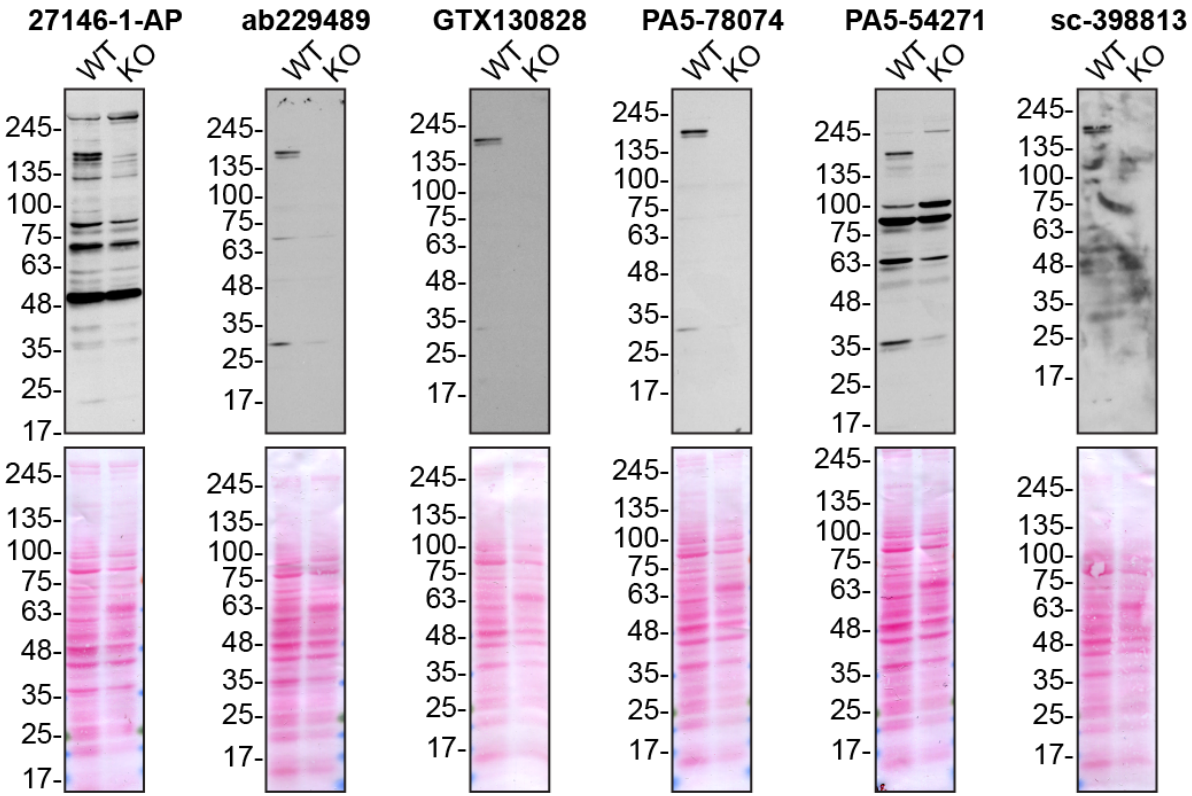

Figure 1: Nek1 antibody screening by immunoblot

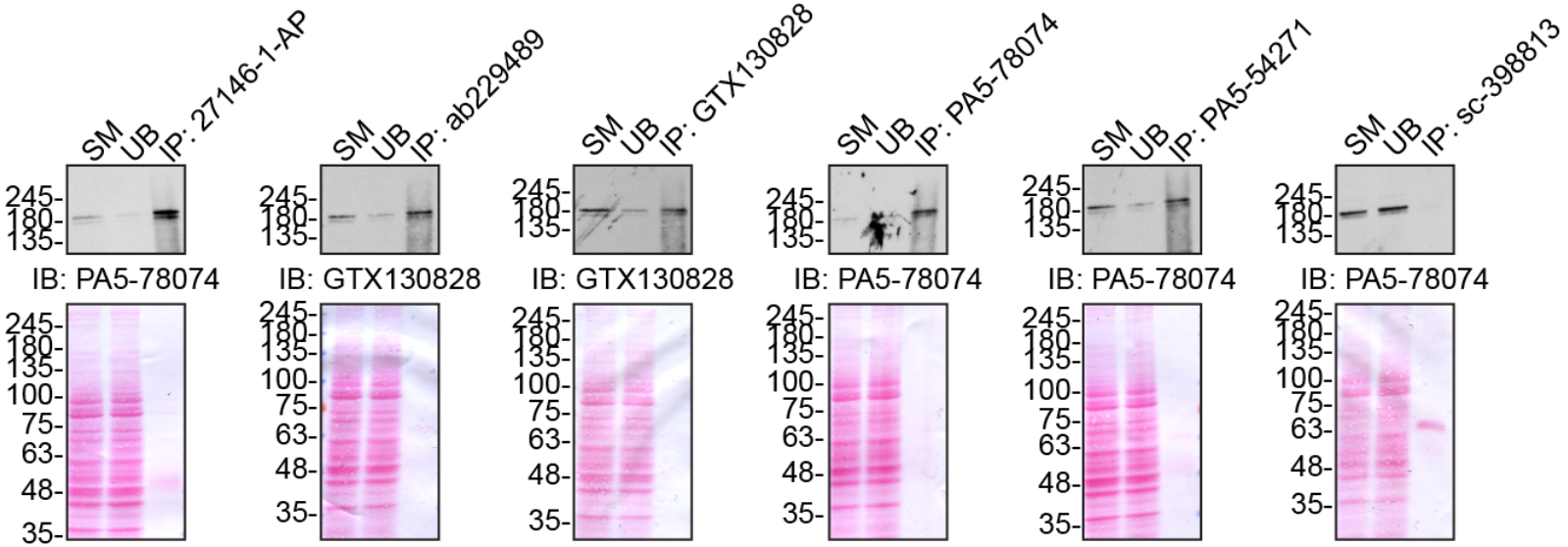

Figure 2: Nek1 antibody screening by immunoprecipitation

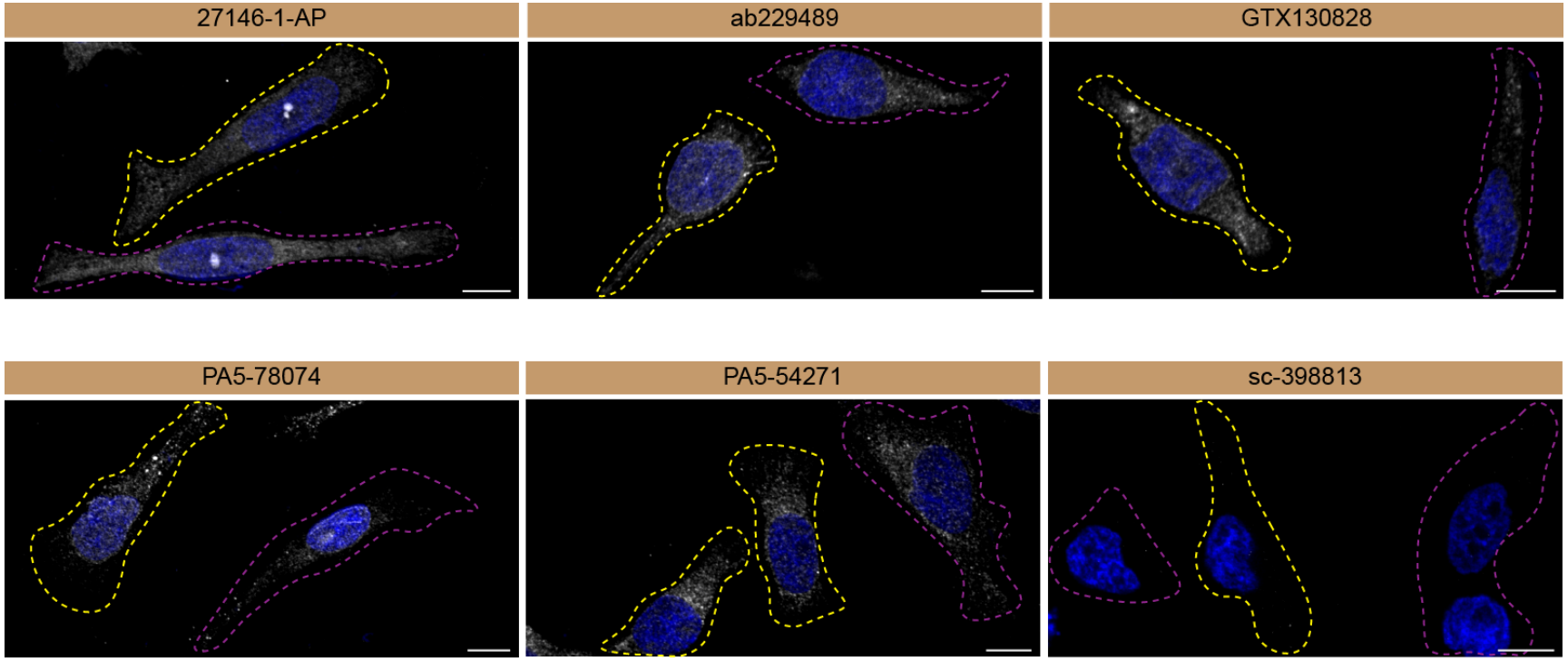

Figure 3: Nek1 antibody screening by immunofluorescence

Materials and methods

Antibodies

All Nek1 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-555-conjugated goat anti-mouse and anti-rabbit secondary antibodies are from Thermo Fisher Scientific (cat. number A21424 and A21429).

CRISPR/Cas9 genome editing

Cell lines used are listed in Table 2. HeLa *NEK1* KO clone was generated with low passage cells using an open-access protocol available on Zenodo.org: https://zenodo.org/record/3738361#.YIyeDu2SlaR. Two guide RNAs were used to introduce a STOP codon in the *NEK1* gene (sequence guide 1: GAGACUAGUACAGGCCUGUU, sequence guide 2: AGAAACGCUGGUCCAAAUCC).

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).

Antibody screening by immunoblot

HeLa (WT and *NEK1* KO) 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 5-16% 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 immunoprecipitation

Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 ul of PBS with 0,01% triton X-100 in a microcentrifuge tube, together with 30µl 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.

HeLa WT 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. Lysates are rocked 30 min at 4°C and spun at 110,000xg for 15 min at 4°C. One ml aliquots at 1.0 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 1.0 ml of HEPES lysis buffer and processed for SDS-PAGE and immunoblot on a 5-16% acrylamide gel.

Antibody screening by immunofluorescence

HeLa WT and *NEK1* KO were labelled with a green and a deep red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific (cat. number C2925 and C34565). WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator. 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 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary Nek1 antibodies O/N at 4°C. Cells were then washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature with DAPI. 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 Zeiss LSM 880 laser scanning confocal microscope equipped with a Plan-Apo 40x oil objective (NA = 1.40). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single focal plane. Figures were assembled with Adobe Illustrator.