Product Datasheet

PINK1 Antibody (8E10.1D6) - BSA Free
NBP2-36488

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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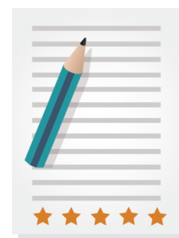


NBP2-36488

PINK1 Antibody (8E10.1D6) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	8E10.1D6
Preservative	0.05% Sodium Azide
Isotype	IgG2b Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	62.7 kDa

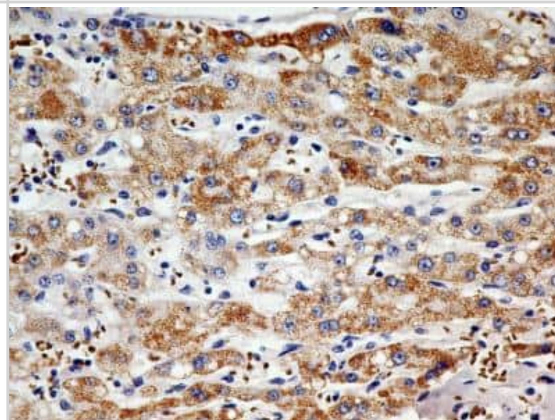
Product Description	
Host	Mouse
Gene ID	65018
Gene Symbol	PINK1
Species	Human, Mouse, Rat
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 29486776). Use in Rat reported in scientific literature (PMID:32365512).
Specificity/Sensitivity	Human PINK1 protein sequence (between residues 100-250), only reactive to isoform 1.
Immunogen	PINK1 antibody was developed using a synthetic peptide made to the human PINK1 protein sequence (between residues 100-250). [Swiss-Prot: Q9BXM7]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, SDS-Page, Knockdown Validated
Recommended Dilutions	Western Blot 2-4 ug/ml, Immunohistochemistry 5 ug/ml, Immunocytochemistry/ Immunofluorescence 20-50 ug/ml, Immunohistochemistry-Paraffin 5 ug/ml, SDS-Page reported in scientific literature (PMID 27553674), Knockdown Validated
Application Notes	Unprocessed PINK1 is 63 kDa which undergoes proteolytic processing to generate 55 kDa and 42 kDa cleaved forms, and bands at the mentioned positions may be expected in Western blot application.

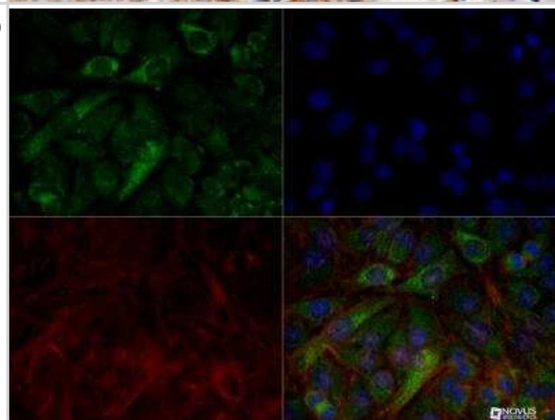
Images

Western Blot: PINK1 Antibody (8E10.1D6) [NBP2-36488] - PINK1 is responsible for CMR-induced cytoplasmic vacuolation.The effect of siRNA-mediated knockdown of PINK1 on CMR-induced changes in the expression of Alix in MDA-MB-231 cells as analyzed by Western blot (left panel) All cells were treated with 6 uM CMR for 48 h. Image collected and cropped by Citeab from the following publication (Chalcomoracin is a potent anticancer agent acting through triggering Oxidative stress via a mitophagy- and paraptosis-dependent mechanism. Sci Rep (2018) licensed under a CC-BY license.

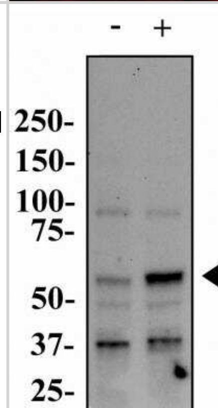
Immunohistochemistry-Paraffin: PINK1 Antibody (8E10.1D6) [NBP2-36488] - Analysis of FFPE tissue section of human hepatocellular carcinoma using PINK1 antibody (clone 8E10.1D6) at 5 ug/ml concentration. The cancer cells developed an expected punctate to granular cytoplasmic staining with no signal in the tumor stroma.

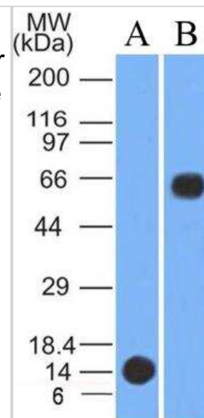
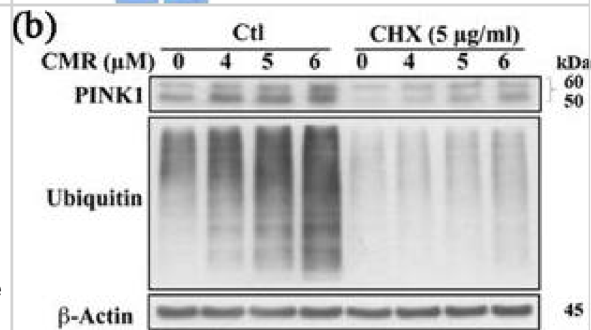
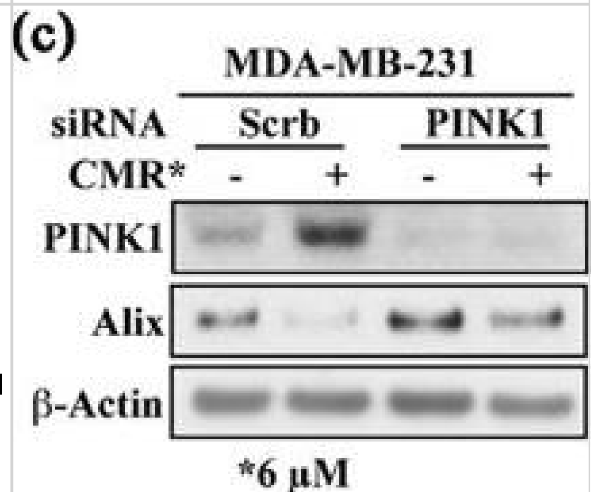


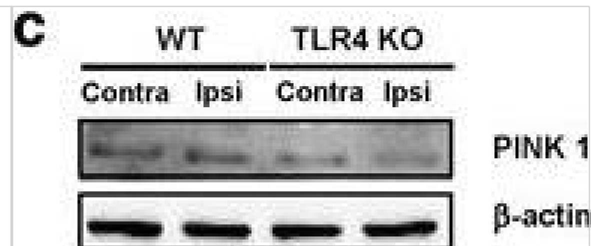
Immunocytochemistry/Immunofluorescence: PINK1 Antibody (8E10.1D6) [NBP2-36488] - HeLa cells were treated with valinomycin (1 uM, 24h) prior to being fixed in 10% buffered formalin for 10 min and permeabilized in 0.1% Triton X-100 in PBS for 10 min. Cells were incubated with NBP2-36488 at 50 ug/ml for 1hr at room temperature, washed 3x in PBS and incubated with Alexa-DyLight-488 anti-mouse secondary antibody. PINK1 (Green) was detected at the mitochondria. Tubulin (Red) was detected using an anti-tubulin antibody with an anti-rabbit DyLight 550 secondary antibody. DNA (Blue) was counterstained with DAPI. Note: mitochondria staining might not be easily observed without treatment with valinomycin or CCCP. Image objective 40x.



Western Blot: PINK1 Antibody (8E10.1D6) [NBP2-36488] - Whole cell protein from HeLa cells treated with or without valinomycin (1 uM, 24h) as indicated was separated by SDS-PAGE on a 7.5% polyacrylamide gel. Protein was transferred to PVDF membrane and probed with 2 ug/ml anti-PINK1 in 1% BSA and detected with an HRP-conjugated anti-mouse secondary antibody using chemiluminescence. PINK1 is seen to be upregulated with treament and with a molecular weight at approximately 60 kDa.



Western Blot: PINK1 Antibody (8E10.1D6) [NBP2-36488] - Analysis of (A) Partial Recombinant Human PINK-1 protein with estimated molecular weight at 13kDa and (B) Human Liver lysate using PINK1 antibody clone 8E10.1D6 at 3ug/ml concentration, molecular weight ~64 kDa.	
Western Blot: PINK1 Antibody (8E10.1D6) - BSA Free [NBP2-36488] - Active protein synthesis is required for CMR-induced cytoplasmic vacuolation. Analysis of CMR-induced cytoplasmic vacuolation in MDA-MB-231 & PC-3 cells by (a) immunocytochemistry & (b) Western blot analysis. Cropped blots were displayed, & original images were included in Fig. S24. The effect of cycloheximide (CHX) on (c) cell viability & (d) cell death was detected by MTT & PI uptake assays. Cells were pre-treated with 5 µg/ml CHX for 1 h. All cells were treated with 6 µM CMR for 48 h. Red: Calreticulin. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29934599), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: PINK1 Antibody (8E10.1D6) - BSA Free [NBP2-36488] - PINK1 is responsible for CMR-induced cytoplasmic vacuolation. (a) Analysis of the effect of ectopic expression of Myc-PINK1 on CMR-induced expression levels of Alix & cytoplasmic vacuolation in LNCaP cells by Western blot (left panel), phase-contrast (middle panel) & immunocytochemistry (right panel). (b) The effect of ectopic expression of Myc-PINK1 on the expression levels of Alix in MDA-MB-231 cells as measured by Western blot. (c) The effect of siRNA-mediated knockdown of PINK1 on CMR-induced changes in the expression of Alix in MDA-MB-231 cells as analyzed by Western blot (left panel) & immunocytochemistry (right panel). Red: Calreticulin. All cells were treated with 6 µM CMR for 48 h. Cropped blots were displayed, & original images were included in Figs S21–S23. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29934599), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	



Western Blot: PINK1 Antibody (8E10.1D6) - BSA Free [NBP2-36488] - Regulation of the mitophagic marker PINK1 in WT & TLR4 KO mice. a PINK1 immunoreactivity was observed in the spinal dorsal horn of CCI mice. PINK1 IR cells were significantly increased in the ipsilateral compared with the contralateral side in WT mice, & no significant difference was found in TLR4 KO mice. Scale bar = 50 μm in A1, A3, A5, & A7. Scale bar = 20 μm in A2, A4, A6, & A8. b The number of PINK1 IR cells was significantly increased in the ipsilateral compared with the contralateral side of WT mice. c Expression of PINK1 was assessed by Western blotting. The PINK1 protein levels were significantly increased in the ipsilateral side compared with the contralateral side in WT mice, & no significant difference was shown in TLR4 KO mice. (D) Quantification by densitometry with Image J. Two-way ANOVA; all the data are shown as mean ± standard deviation, where $*P < 0.05$ denotes a significant difference compared with the control group. d Frozen sections (WT-CCI, POD7) were stained with PINK1 & co-stained with anti-GFAP (A1–4), anti-iba-1 (B1–4), & anti-NeuN antibodies (C1–4). A4, B4 & C4 is rectangular magnification of merged A3, B3 & C3, respectively. Scale bar = 50 μm in A, B, C1–3. Scale bar = 20 μm in A, B, C4 Image collected & cropped by CiteAb from the following publication (https://molecularbrain.biomedcentral.com/articles/10.1186/s13041-018-0354-y), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Publications

Meng Q, Zaharieva EK, Sasatani M, Kobayashi J. Possible relationship between mitochondrial changes and oxidative stress under low dose-rate irradiation Redox Report 2021-08-26 [PMID: 34435550]

Roberta Tufi, Emily H Clark, Tamaki Hoshikawa, Christiana Tsagkaraki, Jack Stanley, Kunitoshi Takeda, James M Staddon, Thomas Briston High-content phenotypic screen to identify small molecule enhancers of Parkin-dependent ubiquitination and mitophagy. SLAS discovery : advancing life sciences R & D 2023-04-25 [PMID: 36608804]

Di Rienzo M, Romagnoli A, Ciccosanti F Et al. AMBRA1 regulates mitophagy by interacting with ATAD3A and promoting PINK1 stability Autophagy 2021-11-19 [PMID: 34798798] (ICC/IF, WB, Human)

Greene A W, Grenier K et al. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. EMBO Rep 2012-01-04 [PMID: 22354088] (WB, Human)

Shin N, Shin HJ, Yi Y et al. p66shc siRNA-Encapsulated PLGA Nanoparticles Ameliorate Neuropathic Pain Following Spinal Nerve Ligation Polymers (Basel) 2020-04-29 [PMID: 32365512] (WB, Rat)

Tan WJT, Song L, Li Y et al. Novel Role of the Mitochondrial Protein Fus1 in Protection from Premature Hearing Loss via Regulation of Oxidative Stress and Nutrient and Energy Sensing Pathways in the Inner Ear Antioxid Redox Signal. 2017-09-09 [PMID: 28135838] (WB, Mouse)

Han H, Chou CC, Li R et al. Chalcomoracin is a potent anticancer agent acting through triggering Oxidative stress via a mitophagy- and paraptosis-dependent mechanism Sci Rep 2018-06-22 [PMID: 29934599] (WB, Human)

Piao Y, Gwon DH, Kang DW et al. TLR4-mediated autophagic impairment contributes to neuropathic pain in chronic constriction injury mice. Mol Brain. 2018-02-27 [PMID: 29486776] (IHC-P, Mouse)

Details:
Mouse monoclonal PINK1 antibody from Novus was used to examine differences in expression of PINK 1 IR cells in the ipsilateral and contralateral side in WT mice vs TLR4 KO mice

Kim MJ, Hwang JW, Yun CK, Lee Y. Delivery of exogenous mitochondria via centrifugation enhances cellular metabolic function. Sci Rep. 2018-02-20 [PMID: 29463809] (WB, Human)

Juyeon K, Park JH, Park YS, Koh HC. PPAR-gamma activation attenuates deltamethrin-induced apoptosis by regulating cytosolic PINK1 and inhibiting mitochondrial dysfunction. Toxicol. Lett. 2016-08-20 [PMID: 27553674]

Procedures

Western Blot protocol for PINK1 Antibody (NBP2-36488)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute anti-PINK1 (8E10.1D6) primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunocytochemistry/Immunofluorescence protocol for PINK1 Antibody (NBP2-36488)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry-Paraffin protocol for PINK1 Antibody (NBP2-36488)

1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
 a. Immerse in 100% ethanol with 2 changes for 5 minutes each
 b. Immerse in 95% ethanol with 2 changes for 5 minutes each
 c. Immerse in 90% ethanol for 5 minutes
 d. Immerse in 70% ethanol for 5 minutes
 e. Immerse in 50% ethanol for 5 minutes
 f. Immerse in distilled water for 5 minutes
3. Antigen Retrieval (Microwave Method):
 a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
 b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
 c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
4. Quenching of Endogenous Peroxidase:
 a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
 b. Wash the slides in TBST 3 times, 3 minutes each.
5. Protein Blocking:
 a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
 b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
6. Primary Antibody:
 a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
 b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
 c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
7. Probe (Secondary Reagent):
 a. Incubate with MACH 1 Mouse probe for 15 minutes at RT.
 b. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
 c. Wash the slides with TBST 4 times, 5 minutes each
8. Chromogen:
 a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
 b. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
 c. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
9. Counter stain:
 a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
 b. Wash in deionized water for 1-2 minutes to clear the extra stain.
 c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
10. Dehydrate the sections in increasing grades of alcohols:
 a. 50% alcohol for 1 minute
 b. 70% for 1 minute
 c. 90% for 1 minute
 d. 95% for 1 minute
 e. 100% for 1 minute
 f. Xylene with 2 changes for 2 minutes each
11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

NOTE:- This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.

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Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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