abcam

Product datasheet

Anti-PARK7/DJ1 antibody [EP2815Y] ab76008

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-PARK7/DJ1 antibody [EP2815Y]
Description	Rabbit monoclonal [EP2815Y] to PARK7/DJ1
Host species	Rabbit
Specificity	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** WB, IP, IHC-P, Flow Cyt (Intra), ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human PARK7/DJ1 aa 1-100 (N terminal). Database link: **Q99497**
Positive control	WB: Jurkat, HeLa, NIH3T3 or 293T cell lysate. Human fetal brain; Human brain nuclear fraction tissue lysate; Mouse brain and Rat brain tissue lysates. IHC-P: Human Lung and Brain tissue. ICC/IF: PANC-1 and Jurkat cell lines. Flow Cyt (intra): HepG2 cells. IP: Mouse brain lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP2815Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab76008 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (2)	1/5000. Predicted molecular weight: 20 kDa. **For unpurified use at 1/10000 - 1/20000.**
IP		1/20.
IHC-P		1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. Perform heat mediated antigen retrieval using 0.01M Sodium Citrate Buffer, pH 6.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100
ICC/IF	★★★★☆ (1)	1/50 - 1/500.

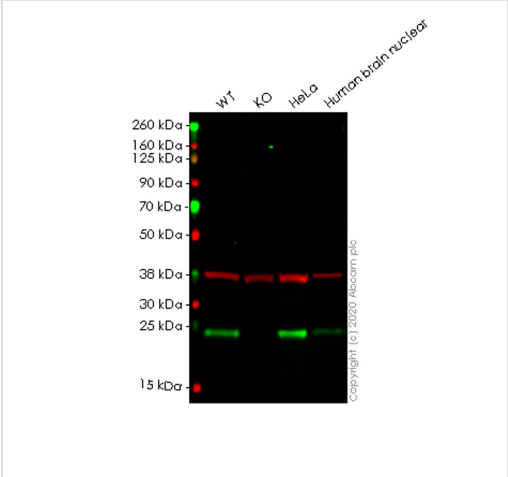
Target

Function Protects cells against oxidative stress and cell death. Plays a role in regulating expression or stability of the mitochondrial uncoupling proteins SLC25A14 and SLC25A27 in dopaminergic neurons of the substantia nigra pars compacta and attenuates the oxidative stress induced by calcium entry into the neurons via L-type channels during pacemaking. Eliminates hydrogen peroxide and protects cells against hydrogen peroxide-induced cell death. May act as an atypical peroxiredoxin-like peroxidase that scavenges hydrogen peroxide. Following removal of a C-terminal peptide, displays protease activity and enhanced cytoprotective action against oxidative stress-induced apoptosis. Stabilizes NFE2L2 by preventing its association with KEAP1 and its subsequent ubiquitination. Binds to OTUD7B and inhibits its deubiquitinating activity. Enhances RELA nuclear translocation. Binds to a number of mRNAs containing multiple copies of GG or CC motifs and partially inhibits their translation but dissociates following oxidative stress. Required for correct mitochondrial morphology and function and for autophagy of dysfunctional mitochondria. Regulates astrocyte inflammatory responses. Acts as a positive regulator of androgen receptor-dependent transcription. Prevents aggregation of SNCA. Plays a role in fertilization. Has no proteolytic activity. Has cell-growth promoting activity and transforming activity. May function as a redox-sensitive chaperone.

Tissue specificity Highly expressed in pancreas, kidney, skeletal muscle, liver, testis and heart. Detected at slightly

	lower levels in placenta and brain. Detected in astrocytes, Sertoli cells, spermatogonia, spermatids and spermatozoa.
Involvement in disease	Defects in PARK7 are the cause of Parkinson disease type 7 (PARK7) [MIM:606324]. A neurodegenerative disorder characterized by resting tremor, postural tremor, bradykinesia, muscular rigidity, anxiety and psychotic episodes. PARK7 has onset before 40 years, slow progression and initial good response to levodopa. Some patients may show traits reminiscent of amyotrophic lateral sclerosis-parkinsonism/dementia complex (Guam disease).
Sequence similarities	Belongs to the peptidase C56 family.
Post-translational modifications	Sumoylated on Lys-130 by PIAS2 or PIAS4; which is enhanced after ultraviolet irradiation and essential for cell-growth promoting activity and transforming activity. Cys-106 is easily oxidized to sulfinic acid. Undergoes cleavage of a C-terminal peptide and subsequent activation of protease activity in response to oxidative stress.
Cellular localization	Cytoplasm. Nucleus. Mitochondrion. Under normal conditions, located predominantly in the cytoplasm and, to a lesser extent, in the nucleus and mitochondrion. Translocates to the mitochondrion and subsequently to the nucleus in response to oxidative stress and exerts an increased cytoprotective effect against oxidative damage. Detected in tau inclusions in brains from neurodegenerative disease patients.

Images



Western blot - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

All lanes : Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 2 : PARK7 knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Human brain nuclear fraction tissue lysate

Lysates/proteins at 20 µg per lane.

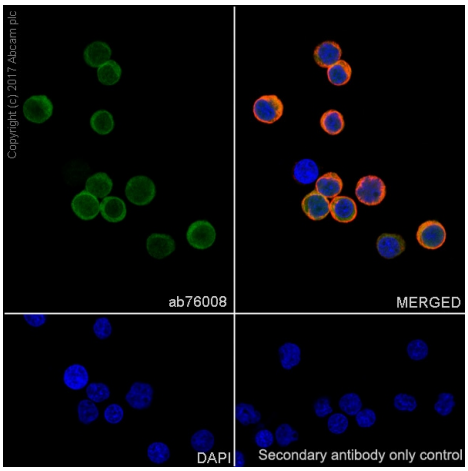
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 20 kDa
Observed band size: 24 kDa

Lanes 1-4: Merged signal (red and green). Green - ab76008

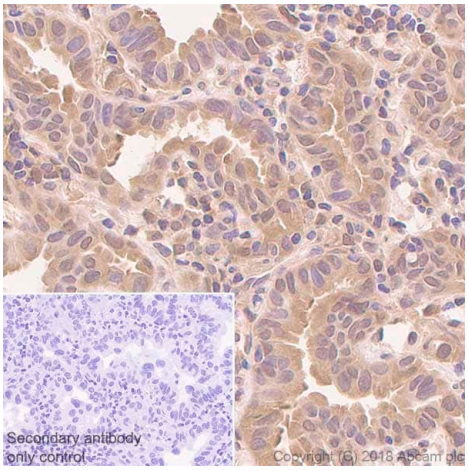
observed at 24 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab76008 Anti-PARK7/DJ1 antibody [EP2815Y] was shown to specifically react with PARK7/DJ1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266338** (knockout cell lysate **ab257016**) was used. Wild-type and PARK7/DJ1 knockout samples were subjected to SDS-PAGE. ab76008 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



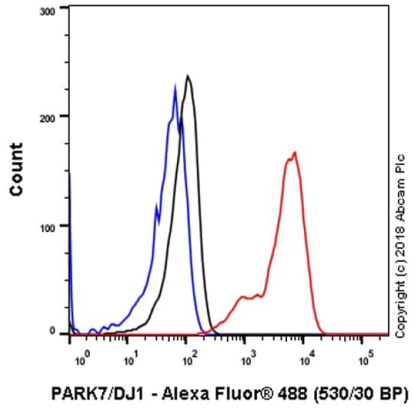
Immunocytochemistry/ Immunofluorescence - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

Immunocytochemistry/ Immunofluorescence analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling PARK7/DJ1 with Purified ab76008 at 1:500 dilution (0.2 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



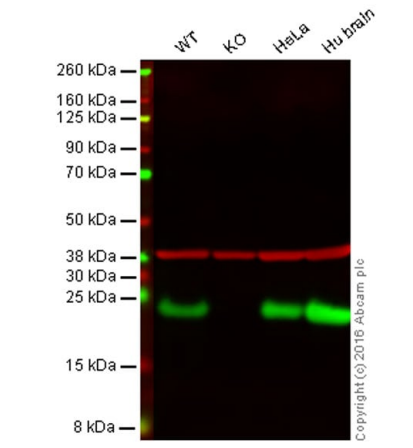
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human lung cancer tissue sections labeling PARK7/DJ1 with Purified ab76008 at 1:1000 dilution (0.11 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use)was used as the secondary antibody. Negative control:PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Flow Cytometry (Intracellular) - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling PARK7/DJ1 with Purified ab76008 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

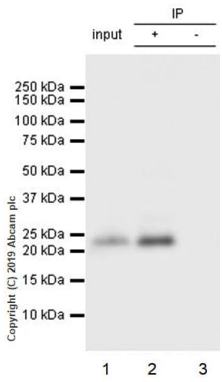


Western blot - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PARK7/DJ1 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Human brain tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab76008 observed at 24 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

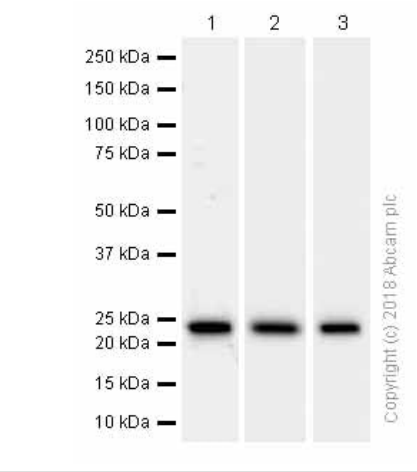
ab76008 was shown to specifically react with PARK/DJ1 in wild-type HAP1 cells. No band was observed when PARK/DJ1 knockout samples were used. Wild-type and PARK/DJ1 knockout samples were subjected to SDS-PAGE. ab76008 and **ab8245** (loading control to GAPDH) were both diluted 1/10,000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG

H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 h at room temperature before imaging.



Immunoprecipitation - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

ab76008 (purified) at 1:20 dilution (0.5µg) immunoprecipitating PARK7/DJ1 in Mouse brain lysate.
Lane 1 (input): Mouse brain lysate 10µg
Lane 2 (+): ab76008 & Mouse brain lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab76008 in Mouse brain lysate
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

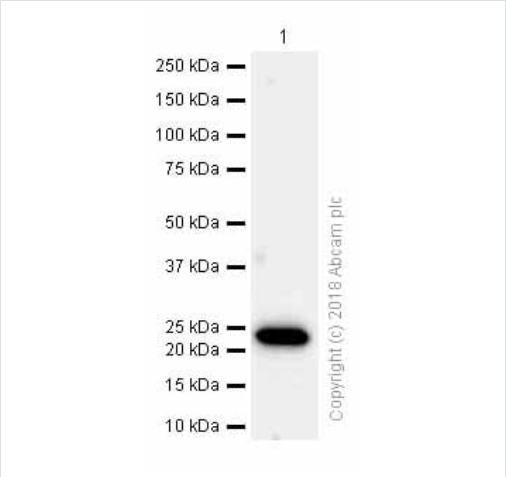
All lanes : Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008) at 1/5000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : Mouse brain lysates
Lane 3 : Rat brain lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 20 kDa
Observed band size: 23 kDa



Western blot - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

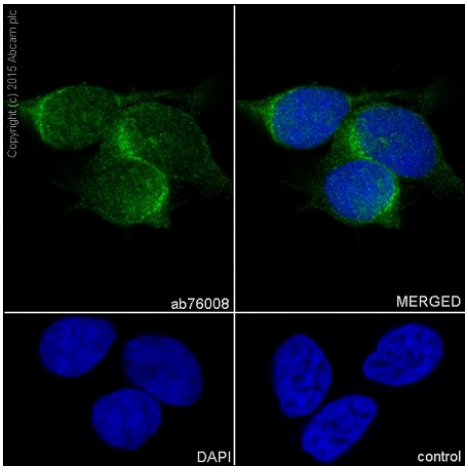
Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008) at 1/5000 dilution (Purified) + Human fetal brain lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 20 kDa

Observed band size: 23 kDa

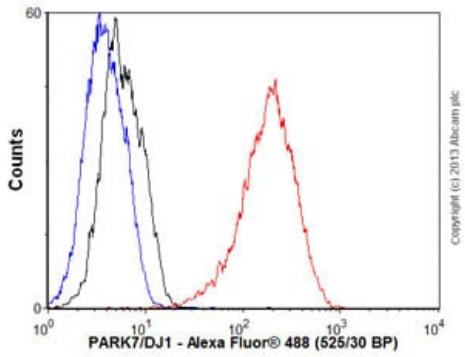


Immunocytochemistry/ Immunofluorescence - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 (human breast carcinoma) labelling PARK7/DJ1 with purified ab76008 at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only

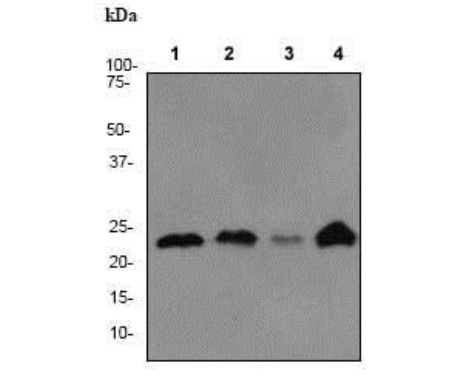
This image was generated using the unpurified version of the product.



Flow Cytometry (Intracellular) - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

Overlay histogram showing HepG2 cells stained with ab76008 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76008, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HepG2 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This image was generated using the unpurified version of the product.



Western blot - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

All lanes : Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008) at 1/20000 dilution

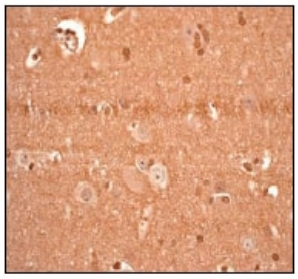
Lane 1 : Jurkat cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : NIH3T3 cell lysate
Lane 4 : 293T cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-rabbit HRP at 1/1000 dilution

Predicted band size: 20 kDa
Observed band size: 23 kDa

This image was generated using the unpurified version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

ab76008, at 1/250 dilution, staining PARK7/DJ1 in human brain by immunohistochemistry using paraffin-embedded tissue.

This image was generated using the unpurified version of the product.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-PARK7/DJ1 antibody [EP2815Y] (ab76008)

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