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Product datasheet

Anti-Parkin antibody [PRK8] ab77924

KO VALIDATED

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Overview

Product name	Anti-Parkin antibody [PRK8]
Description	Mouse monoclonal [PRK8] to Parkin
Host species	Mouse
Tested applications	**Suitable for:** IHC-Fr, Flow Cyt, WB, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, Drosophila melanogaster
Immunogen	Recombinant full length protein corresponding to Human Parkin.
Epitope	The epitope is the second ring domain (aa 399-465).
Positive control	WB: SH-SY5Y whole cell lysate. Human, mouse and rat brain tissue lysates. Wild-type HAP1 whole cell lysate. Flow Cyt: SH-SY5Y cells.
General notes	This antibody clone is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	PRK8
Isotype	IgG2b
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab77924** 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
IHC-Fr		Use at an assay dependent concentration. PubMed: 20689587
Flow Cyt		Use 1µg for 10^6 cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
WB	★★★★☆	Use a concentration of 5 µg/ml. Detects a band of approximately 55 kDa (predicted molecular weight: 52 kDa). Abcam recommends using 1-3% Milk as the blocking agent. Higher percentage blocking solutions may not give optimal results.
IP	★★★★★	Use at an assay dependent concentration.

Target

Function

Functions within a multiprotein E3 ubiquitin ligase complex, catalyzing the covalent attachment of ubiquitin moieties onto substrate proteins, such as BCL2, SYT11, CCNE1, GPR37, STUB1, a 22 kDa O-linked glycosylated isoform of SNCAIP, SEPT5, ZNF746 and AIMP2. Mediates monoubiquitination as well as 'Lys-48'-linked and 'Lys-63'-linked polyubiquitination of substrates depending on the context. Participates in the removal and/or detoxification of abnormally folded or damaged protein by mediating 'Lys-63'-linked polyubiquitination of misfolded proteins such as PARK7: 'Lys-63'-linked polyubiquitinated misfolded proteins are then recognized by HDAC6, leading to their recruitment to aggresomes, followed by degradation. Mediates 'Lys-63'-linked polyubiquitination of SNCAIP, possibly playing a role in Lewy-body formation. Mediates monoubiquitination of BCL2, thereby acting as a positive regulator of autophagy. Promotes the autophagic degradation of dysfunctional depolarized mitochondria. Mediates 'Lys-48'-linked polyubiquitination of ZNF746, followed by degradation of ZNF746 by the proteasome; possibly playing a role in role in regulation of neuron death. Limits the production of reactive oxygen species (ROS). Loss of this ubiquitin ligase activity appears to be the mechanism underlying pathogenesis of PARK2. May protect neurons against alpha synuclein toxicity, proteasomal dysfunction, GPR37 accumulation, and kainate-induced excitotoxicity. May play a role in controlling neurotransmitter trafficking at the presynaptic terminal and in calcium-dependent exocytosis. Regulates cyclin-E during neuronal apoptosis. May represent a tumor suppressor gene.

Tissue specificity

Highly expressed in the brain including the substantia nigra. Expressed in heart, testis and skeletal muscle. Expression is down-regulated or absent in tumor biopsies, and absent in the brain of PARK2 patients. Overexpression protects dopamine neurons from kainate-mediated apoptosis. Found in serum (at protein level).

Pathway

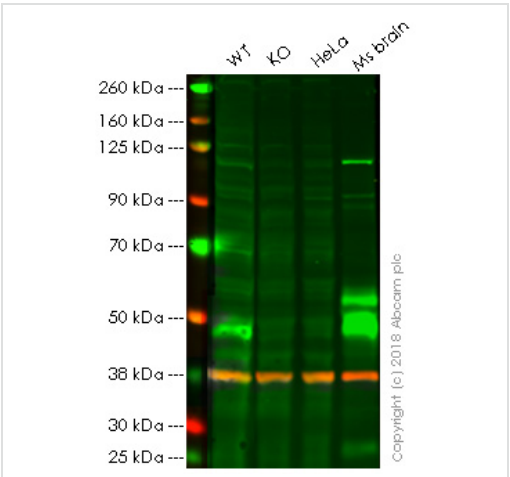
Protein modification; protein ubiquitination.

Involvement in disease

Defects in PARK2 are a cause of Parkinson disease (PARK) [MIM:168600]. A complex neurodegenerative disorder characterized by bradykinesia, resting tremor, muscular rigidity and postural instability. Additional features are characteristic postural abnormalities, dysautonomia, dystonic cramps, and dementia. The pathology of Parkinson disease involves the loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies (intraneuronal accumulations of aggregated proteins), in surviving neurons in various areas of the brain. The

	disease is progressive and usually manifests after the age of 50 years, although early-onset cases (before 50 years) are known. The majority of the cases are sporadic suggesting a multifactorial etiology based on environmental and genetic factors. However, some patients present with a positive family history for the disease. Familial forms of the disease usually begin at earlier ages and are associated with atypical clinical features. Defects in PARK2 are the cause of Parkinson disease type 2 (PARK2) [MIM:600116]; also known as early-onset parkinsonism with diurnal fluctuation (EPDF) or autosomal recessive juvenile Parkinson disease (PDJ). A neurodegenerative disorder characterized by bradykinesia, rigidity, postural instability, tremor, and onset usually befor 40. It differs from classic Parkinson disease by early DOPA-induced dyskinesia, diurnal fluctuation of the symptoms, sleep benefit, dystonia and hyper-reflexia. Dementia is absent. Pathologically, patients show loss of dopaminergic neurons in the substantia nigra, similar to that seen in Parkinson disease; however, Lewy bodies (intraneuronal accumulations of aggregated proteins) are absent. Note=Defects in PARK2 may be involved in the development and/or progression of ovarian cancer.
Sequence similarities	Belongs to the RBR family. Parkin subfamily. Contains 1 IBR-type zinc finger. Contains 2 RING-type zinc fingers. Contains 1 ubiquitin-like domain.
Domain	The ubiquitin-like domain binds the PSMD4 subunit of 26S proteasomes.
Post-translational modifications	Auto-ubiquitinates in an E2-dependent manner leading to its own degradation. Also polyubiquitinated by RNF41 for proteasomal degradation. S-nitrosylated. The inhibition of PARK2 ubiquitin E3 ligase activity by S-nitrosylation could contribute to the degenerative process in PD by impairing the ubiquitination of PARK2 substrates.
Cellular localization	Cytoplasm > cytosol. Nucleus. Endoplasmic reticulum. Mitochondrion. Mainly localizes in the cytosol. Co-localizes with SYT11 in neutrites. Co-localizes with SNCAIP in brainstem Lewy bodies. Relocates to dysfunctional mitochondria that have lost the mitochondial membrane potential; recruitement to mitochondria is PINK1-dependent.

Images



Western blot - Anti-Parkin antibody [PRK8] (ab77924)

All lanes : Anti-Parkin antibody [PRK8] (ab77924) at 1/500 dilution

Lane 1 : Wild-type HAP1 whole cell lysate at 40 µg
Lane 2 : PARK2 (Parkin) knockout HAP1 whole cell lysate at 40 µg
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg
Lane 4 : Mouse brain whole cell lysate at 20 µg

Predicted band size: 52 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab77924 observed at 52 kDa. Red - loading control, ab181602, observed at

37 kDa.

ab77924 was shown to specifically react with Parkin in wild-type HAP1 cells as signal was lost in PARK2 (Parkin) knockout cells. Wild-type and PARK2 (Parkin) knockout samples were subjected to SDS-PAGE. ab77924 and ab181602 (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed ab216772 and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

(Parkin expression in HeLa is expected to be negative/low PMID: 14614460 PMID: 12972409)

Western blot - Anti-Parkin antibody [PRK8] (ab77924)

All lanes : Anti-Parkin antibody [PRK8] (ab77924) at 1/2000 dilution

Lane 1 : SH-SY5Y (Human neuroblastoma cell line) Whole Cell Lysate
Lane 2 : Brain (Rat) Tissue Lysate
Lane 3 : Brain (Mouse) Tissue Lysate
Lane 4 : Human brain tissue lysate - total protein (ab29466)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal Secondary Antibody to Mouse IgG - H&L (HRP), pre-adsorbed at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 52 kDa
Additional bands at: 55 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 20 minutes

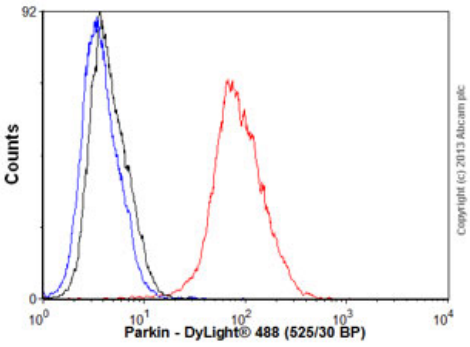
All lanes blocked with 3% milk.

Immunohistochemistry (Frozen sections) - Anti-Parkin antibody [PRK8] (ab77924)

Image from Fett ME et al., PLoS One. 2010 Jul 30;5(7):e11783. Fig 10.; doi:10.1371/journal.pone.0011783; July 30, 2010, PLoS ONE 5(7): e11783.

Immunohistochemical analysis of transgenic Zebrafish expressing human Parkin, staining Parkin with ab77924.

Tissue was fixed with paraformaldehyde and blocked with 10% newborn calf serum with 0.1% Tween for 2 hours. Samples were incubated with primary antibody (1/200) overnight at 4°C. An AlexaFluor®488-conjugated anti-mouse IgG was used as the secondary antibody.



Flow Cytometry - Anti-Parkin antibody [PRK8] (ab77924)

Overlay histogram showing SH-SY5Y cells stained with ab77924 (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 (ab77924, 1/100 dilution) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions.

Acquisition of >5,000 events was performed.

This antibody gave a positive signal in SH-SY5Y cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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