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Product datasheet

Anti-LRRK2 antibody [MJFF2 (c41-2)] ab133474

敲除验证 重组 RabMAb

★★★★★ 2 Abreviews 106 References 7 图像

概述

产品名称	Anti-LRRK2抗体[MJFF2 (c41-2)]
描述	兔单克隆抗体[MJFF2 (c41-2)] to LRRK2
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, WB, IP
种属反应性	**与反应:** Mouse, Human **预测可用于:** Rat
免疫原	Recombinant fragment within Human LRRK2 aa 950 to the C-terminus. The exact sequence is proprietary. Database link: **Q5S007**
阳性对照	WB: A549 and MEF cell lysates; HEK293 cells transfected with LRRK2 cell lysate. ICC/IF: Neuro-2a cells. IHC-P: SNc of 1 y-old Rgs6-/- mice; Mouse brain tissue IP: Mouse cerebral cortex, A549 .
常规说明	Well-characterized antibodies to efficiently detect and purify LRRK2 protein are a critical need in the Parkinson's Disease (PD) research community. To help accelerate LRRK2 research, The Michael J. Fox Foundation (MJFF), working with Abcam, has generated unique and high quality LRRK2 rabbit monoclonal antibodies to be widely available for PD research community. LRRK2 (Leucine-rich repeat kinase 2, dardarin) is a protein kinase belonging to the ROCO family, which is defined by the presence of a ROC (Ras/GTPase of complex proteins) domain and COR (C-terminal of Roc) region. LRRK2 exhibits kinase activity whereby it can undergo autophosphorylation and can phosphorylate generic substrates. In addition, the GTPase domain of LRRK2 can mediate GDP (guanosine-5′-diphosphate)/GTP (guanosine-5′-triphosphate) binding as well as GTP hydrolysis. LRRK2 is mutated in a significant number of Parkinson's disease (PD) patients. Mutations in this gene account for 4% of PD, and are observed in 1% of sporadic PD patients. Clinical symptoms of patients carrying PD-associated mutations of LRRK2 are indistinguishable from typical sporadic PD. The spectra of neuropathological features of PARK8 (type 8), the type corresponding to LRRK2, is broad and appears to encompass those associated with other familial PD cases such as PARK1 (alpha-synuclein) and PARK2 (Parkin). Patients with this gene mutation have typical relatively late onset Parkinsonism with features comparable with idiopathic PD; symptoms include asymmetric rest tremor, bradykinesia, rigidity, and a good response to

3,4-dihyroxy-l-phenylalanine (l-DOPA). The pathology of cases with LRRK2 mutations is pleomorphic.

For more characterization data and protocols using this LRRK2 Antibody, please refer to Davies, et al. 2013. Biochemical J 453(1):101-113 [PMID: 23560750]
Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). Or search our wide range of secondary antibodies for use with your experiment.

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**.

This antibody was developed with support from The Michael J. Fox Foundation.

THE MICHAEL J. FOX FOUNDATION
FOR PARKINSON'S RESEARCH

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	MJFF2 (c41-2)
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab133474于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

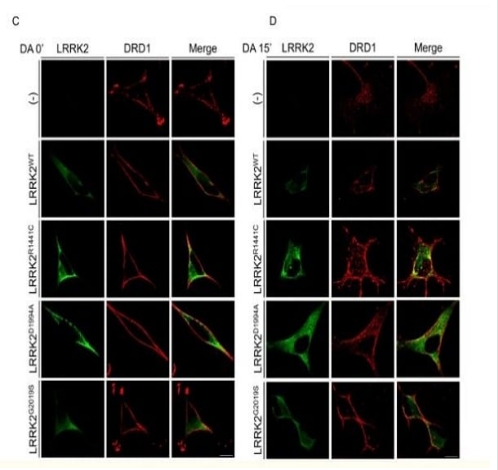
应用	Ab评论	说明
ICC/IF		1/50.
WB	★★★★★ (2)	1/10000 - 1/50000. Predicted molecular weight: 286 kDa.

应用	Ab评论	说明
IP		Use at an assay dependent concentration. (2-5 µg)

靶标

功能	Positively regulates autophagy through a calcium-dependent activation of the CaMKK/AMPK signaling pathway. The process involves activation of nicotinic acid adenine dinucleotide phosphate (NAADP) receptors, increase in lysosomal pH, and calcium release from lysosomes. Together with RAB29, plays a role in the retrograde trafficking pathway for recycling proteins, such as mannose 6 phosphate receptor (M6PR), between lysosomes and the Golgi apparatus in a retromer-dependent manner. Regulates neuronal process morphology in the intact central nervous system (CNS). Plays a role in synaptic vesicle trafficking. Phosphorylates PRDX3. Has GTPase activity. May play a role in the phosphorylation of proteins central to Parkinson disease.
组织特异性	Expressed in the brain. Expressed in pyramidal neurons in all cortical laminae of the visual cortex, in neurons of the substantia nigra pars compacta and caudate putamen (at protein level). Expressed throughout the adult brain, but at a lower level than in heart and liver. Also expressed in placenta, lung, skeletal muscle, kidney and pancreas. In the brain, expressed in the cerebellum, cerebral cortex, medulla, spinal cord occipital pole, frontal lobe, temporal lobe and putamen. Expression is particularly high in brain dopaminoceptive areas.
疾病相关	Parkinson disease 8
序列相似性	Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. Contains 12 LRR (leucine-rich) repeats. Contains 1 protein kinase domain. Contains 1 Roc domain. Contains 7 WD repeats.
结构域	The seven-bladed WD repeat region is critical for synaptic vesicle trafficking and mediates interaction with multiple vesicle-associated presynaptic proteins. The Roc domain mediates homodimerization and regulates kinase activity.
翻译后修饰	Autophosphorylated.
细胞定位	Membrane. Cytoplasm. Perikaryon. Mitochondrion. Golgi apparatus. Cell projection, axon. Cell projection, dendrite. Endoplasmic reticulum. Cytoplasmic vesicle, secretory vesicle, synaptic vesicle membrane. Endosome. Lysosome. Mitochondrion outer membrane. Mitochondrion inner membrane. Mitochondrion matrix. Predominantly associated with intracytoplasmic vesicular and membranous structures (By similarity). Localized in the cytoplasm and associated with cellular membrane structures. Predominantly associated with the mitochondrial outer membrane of the mitochondria. Colocalized with RAB29 along tubular structures emerging from Golgi apparatus. Localizes in intracytoplasmic punctate structures of neuronal perikarya and dendritic and axonal processes.

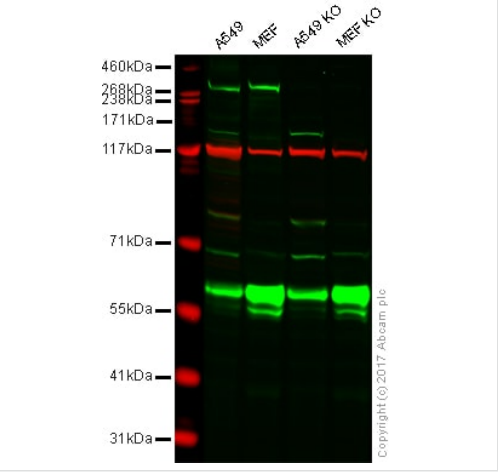
图片



Immunocytochemistry/ Immunofluorescence - Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474)

Evaluation of DRD1 intracellular and extracellular level by BPA upon dopamine treatment in SH-SY5Y-DRD1 cells untransduced or transduced by WT or G2019S LRRK2

(C and D) DRD1 localization at basal conditions (C) and upon 15 minutes (D) of dopamine treatment of SH-SY5Y-DRD1 cells transduced or not by alpha-synuclein WT or A53T. After agonist treatment the cells were fixed and incubated with the different primary antibodies (anti-FLAG for DRD1 and ab133474) and with Alexa647-conjugated secondary antibody (red) or Alexa488-conjugated secondary antibody (green) for DRD1 or LRRK2 respectively. Scale bars = 10µm.



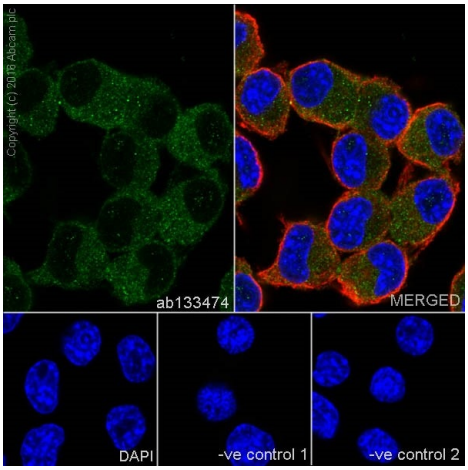
Western blot - Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474)

Lane 1: Wild type A549 whole cell lysate (20 µg)
Lane 2: Wild type MEF whole cell lysate (20 µg)
Lane 3: LRRK2 knockout A549 whole cell lysate (20 µg)
Lane 4: LRRK2 knockout MEF whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab133474 observed at 286 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

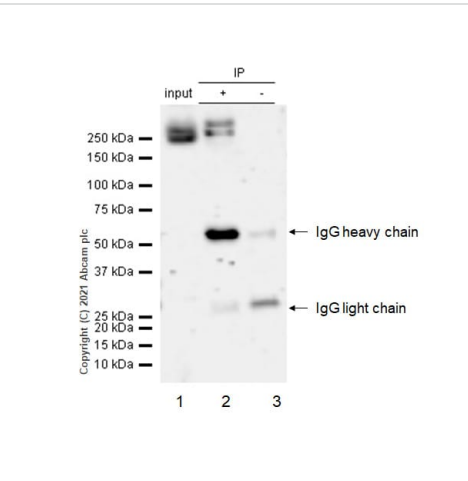
ab133474 was shown to recognize LRRK2 in wild type A549 and MEF cells along with additional cross reative bands. Whilst signal was not seen in LRRK2 knockout cells. Wild-type and LRRK2 knockout samples were subjected to SDS-PAGE. Ab133474 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 10000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Wild-type and LRRK2 knockout MEF and A549 cells were provide as a generous gift from Professor Dario Alessi, MRC Protein Phosphorylation and Ubiquitination Unit (University of Dundee).



Immunocytochemistry/ Immunofluorescence - Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474)

ab133474 staining LRRK2 in Neuro-2a (mouse neuroblastoma cell line) cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody (1/500). **ab150077** was used as the secondary antibody (1/1000). Tubulin stained using **ab7291** (1/1000) and **ab150120** (1/1000) as the secondary. Nuclear counter stained with DAPI.



Immunoprecipitation - Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474)

LRRK2 was immunoprecipitated from 0.35 mg A549 (Human lung carcinoma epithelial cell) whole cell lysate 10 µg with 133474 at 1/60 dilution (2µg). VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

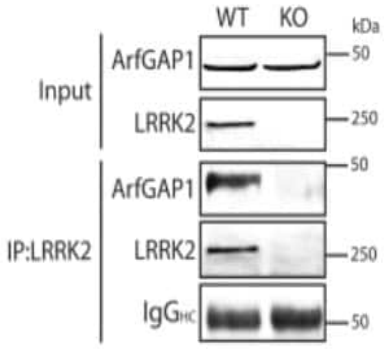
Lane 1: A549 (Human lung carcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: ab133474 IP in A549 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab133474 in A549 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Fresh lysate should be used to minimize protein degradation.

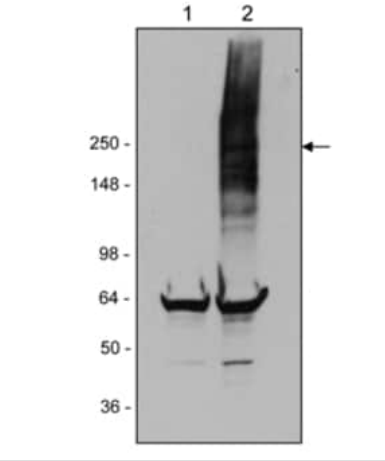


Immunoprecipitation - Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474)

Stafa K. et al., PLoS Genet. 2012;8(2):e1002526. Fig 1 doi: 10.1371/journal.pgen.1002526. Reproduced under the Creative Commons license

Immunoprecipitation to verify the interaction of LRRK2 and ArfGAP1 *in vivo*. LRRK2 interacts with ArfGAP1 in brain extracts derived from wild-type mice following immunoprecipitation with ab133474, a LRRK2-specific monoclonal antibody (MJFF-2/c41-2), whereas ArfGAP1 is not immunoprecipitated in extracts derived from LRRK2 knockout mice

Protein extracts were prepared from the cerebral cortex of adult wild-type and LRRK2 knockout mice (with targeted deletion of exon 41 of the LRRK2 gene) by homogenization in TNE buffer (10 mM Tris-HCL pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1× phosphatase inhibitor cocktail 1 and 2, 1× Complete Mini protease inhibitor cocktail). Protein concentration was determined by BCA assay. Brain extracts (10 mg protein) were combined with 50 µl Protein G-Dynabeads pre-incubated with rabbit anti-LRRK2 (5 µg; MJFF2/c41-2; Abcam, Inc.), rabbit anti-ArfGAP1 (3 µg) or rabbit IgG (3 µg) antibodies followed by overnight incubation at 4°C. Dynabead complexes were sequentially washed twice with TNE buffer and twice with TBS buffer (10 mM Tris-HCL pH 7.4, 150 mM NaCl). Immunoprecipitates were eluted by heating at 70°C for 10 min, resolved by SDS-PAGE and subjected to Western blot analysis.



Western blot - Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474)

This image is courtesy of Zhuohua Zhang Lab (Sanford-Burnham Medical Research Institute)

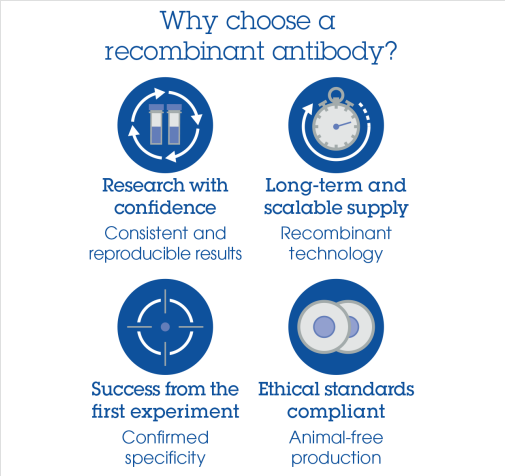
All lanes : Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474) at 1/10000 dilution

Lane 1 : HEK293 cell lysate transfected with 3*Flag vector
Lane 2 : HEK293 cell lysate transfected with 3*Flag full length wild type LRRK2

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 286 kDa



Anti-LRRK2 antibody [MJFF2 (c41-2)] (ab133474)

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