# abcam

## Product datasheet

# Anti-LRRK2 antibody [UDD3 30(12)] ab133518





#### 18 References 7 Images

#### Overview

**Product name** Anti-LRRK2 antibody [UDD3 30(12)]

**Description** Rabbit monoclonal [UDD3 30(12)] to LRRK2

**Host species** Rabbit

Specificity This antibody does not give a positive signal in Human fetal brain. Please contact our Scientific

Support team if you have any questions.

Suitable for: ICC/IF, IHC-P, WB **Tested applications** 

Unsuitable for: IP

Species reactivity Reacts with: Mouse, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control GFP-LRRK2, GFP LRRK2 S910A, GFP LRRK2 S935A, LRRK2 WT MEF, LRRK2 WT MEF

lysate from LRRK2-IN1 treated cells, Lymphoblastoid lysates and Lymphoblastoid lysate from

LRRK2-IN1 treated cells.

General notes In recent years, a critical need in the Parkinson's Disease (PD) research community has been

> access to well-characterized antibodies that can be used to efficiently detect and purify Leucine-Rich Repeat Kinase 2 (LRRK2) protein. The Michael J. Fox Foundation (MJFF) has supported this effort by partnering with Dr. Dario Alessi (MRC Protein Phosphorylation Unit, University of Dundee) to help accelerate LRRK2 research. Dr. Alessi has characterized unique and high quality LRRK2 rabbit monoclonal antibodies, generated by Epitomics, to be made widely available for

PD research community.

LRRK2 (Leucine-rich repeat kinase 2, dardarin) is a multi-domain protein belonging to the ROCO family of proteins that contains a kinase and GTPase domain among its many protein interaction domains. 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. The most common mutation replaces glycine 2019 with a serine that results in increased LRRK2 kinase activity. This indicates that inhibitors of LRRK2 kinase activity might be of therapeutic benefit for the treatment of Parkinson's disease and has stimulated much activity in this field of

research.

The Dundee-MJFF LRRK2[100-500] total antibody will be of great utility in further understanding LRRK2 as it relates to Parkinson's disease. It should be noted this antibody is highly selective and sensitive and can readily be used to monitor LRRK2 protein levels in immunoblot analysis of 2-20 microgram amounts of whole cell extract. The LRRK2[100-500] total antibody recognizes both mouse and human endogenous LRRK2.

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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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

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



## **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.

Stable for 12 months at -20°C.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

**Purity** Protein A purified

Clonality Monoclonal
Clone number UDD3 30(12)

**Isotype** IgG

## **Applications**

## The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab133518 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
ICC/IF		1/200.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.
WB		1/1000 - 1/10000. Predicted molecular weight: 286 kDa.

**Application notes** Is unsuitable for IP.

## **Target**

#### **Function**

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.

## Tissue specificity

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.

## Involvement in disease

Parkinson disease 8

#### Sequence similarities

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.

#### **Domain**

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.

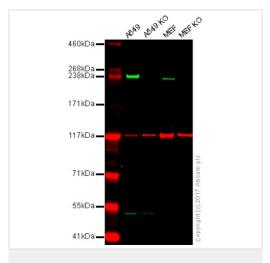
# Post-translational modifications

Autophosphorylated.

## **Cellular localization**

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.

## Images



Western blot - Anti-LRRK2 antibody [UDD3 30(12)] (ab133518)

Lane 1: Wild-type A549 cell lysate (20 µg)

Lane 2: LRRK2 knockout A549 cell lysate (20 µg)

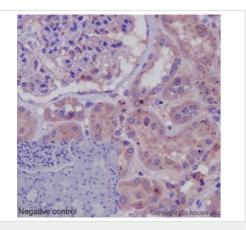
Lane 3: Wild-type MEF cell lysate (20 µg)

Lane 4: LRRK2 knockout MEF cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab133518 observed at 238 kDa. Red - loading control, <u>ab130007</u>, observed at 124 kDa.

ab133518 was shown to specifically react with wild type A549 and MEF cell lines. No band was observed when knock out samples were used. Wild-type and LRRK2 knockout samples were subjected to SDS-PAGE. Ab133518 and <a href="mailto:ab130007">ab130007</a> (loading control to Vinculin) were diluted at 1/500 and 1/10000 dilution respectively and incubated overnight at 4C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

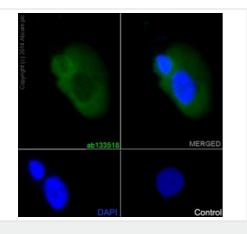
Wild-type and LRRK2 knockout MEF and A549 cells were provide as generous a gift from Professor Dario Alessi, MRC Protein Phosphorylation and Ubiquitination Unit (University of Dundee).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LRRK2 antibody [UDD3 30(12)] (ab133518)

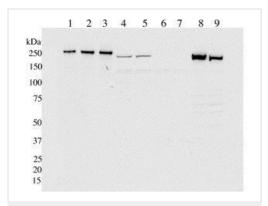
Immunohistochemical staining of paraffin-embedded human kidney with purified ab133518 at a dilution of 1/100. A prediluted HRP polymer for rabbit IgG was used as the secondary and the sample was stained with hematoxylin. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

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



Immunocytochemistry/ Immunofluorescence - Anti-LRRK2 antibody [UDD3 30(12)] (ab133518)

Immunofluorescent staining of SH-SY5Y cells fixed and permeablized with 4% PFA and 0.1% Triton X 100 using purified ab133518 at a dilution of 1/200. An Alexa Fluor<sup>®</sup> 488 goat antirabbit was used as the secondary (ab150077, 1/400) and the sample was stained with DAPI. The negative control is shown in bottom right hand panel - for the negative control, purified ab133518 was used at a dilution of 1/200 followed by an Alexa Fluor<sup>®</sup> 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500.



Western blot - Anti-LRRK2 antibody [UDD3 30(12)] (ab133518)

**All lanes :** Anti-LRRK2 antibody [UDD3 30(12)] (ab133518) at 1/1000 dilution (Unpurified)

Lane 1: GFP-LRRK2 lysate at 5 µg

Lane 2: GFP LRRK2 S910A lysate at 5 µg

Lane 3: GFP LRRK2 S935A lysate at 5  $\mu g$ 

Lane 4: LRRK2 WT MEF lysate at 20 µg

Lane 5: LRRK2 WT MEF lysate from

LRRK2-IN1 treated cells at 20 µg

Lane 6: LRRK2 KO MEF lysate at 20 µg

Lane 7: LRRK2 KO MEF lysate from

LRRK2-IN1 treated cells at 20 µg

Lane 8: Lymphoblastoid lysate at 30 µg

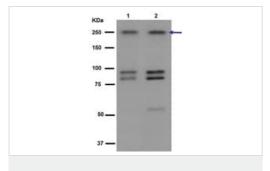
Lane 9: Lymphoblastoid lysate from

LRRK2-IN1 treated cells at 30 µg

## Secondary

All lanes: Goat-anti-rabbit HRP at 1/2000 dilution

Predicted band size: 286 kDa



Western blot - Anti-LRRK2 antibody [UDD3 30(12)] (ab133518)

All lanes: Anti-LRRK2 antibody [UDD3 30(12)] (ab133518) at

1/1000 dilution (Purified)

Lane 1: NIH/3T3 cell lysate Lane 2: RAW264.7 cell lysate

Lysates/proteins at 20 µg per lane.

## Secondary

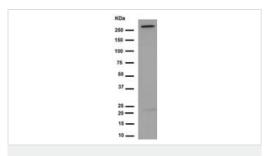
All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 286 kDa

Observed band size: 286 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-LRRK2 antibody [UDD3 30(12)] (ab133518)

Anti-LRRK2 antibody [UDD3 30(12)] (ab133518) at 1/10000 dilution (Purified) + LRRK2 over-expressed 293 cell lysate at 10 μg

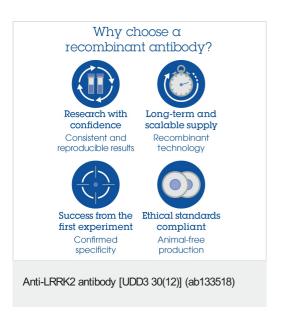
## Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 286 kDa Observed band size: 286 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



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